

**THE EFFECTIVENESS OF
SILVER DIAMINE FLUORIDE ON
REMINERALISATION/
DEMINERALISATION OF
ARTIFICIALLY DEMINERALISED
ENAMEL AND DENTINE LESIONS
*IN VITRO***

Noorhidayah Binti Zainal Aalam

**Submitted in accordance with the requirements for the degree of
Doctor of Paediatric Dentistry**

**The University of Leeds
School of Dentistry
Division of Paediatric Dentistry**

August, 2019

The candidate confirms that the work submitted is his own and that appropriate credit has been given where reference has been made to the work of others.

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Acknowledgement

First and foremost, I would like to pay special appreciation to my supervisor, Dr Marina Malinowski for her vital support and guidance throughout this project, for her patience, motivation, enthusiasm, and immense knowledge. I could not have imagined having a better advisor and mentor for my study. Together with Dr Simon Strafford, without their precious support, it would not be possible to conduct this research and completing this thesis. I would also like to thank Dr Simon Wood, for his advice and assistance in the thesis writing process.

I would like to reserve special thanks to Professor M. Duggal and Professor J. Toumba. Their ideas, encouragement and insightful comments at the beginning of this study made it possible to achieve my goal.

I wish to present my special thanks to the staff and colleagues in the Paediatric Dentistry Department, especially to those who did their best to put me on the right track.

I gratefully acknowledge this wonderful opportunity and funding received towards my doctorate study from the Ministry of Education and University of Malaya, Malaysia.

My most enormous gratitude goes to my beloved parents, who constantly remembered me in their prayers. They have always been supporting me spiritually throughout my life.

And finally, to my dear husband, Firdaus, for his never-ending support and motivation, and without whom, I would not have had the courage to embark on this journey in the first place.

Abstract

To investigate the effect of Silver Diamine Fluoride (SDF), SDF with potassium iodide (SDF/KI) and Fluoride varnish (FV) on progression/regression of artificially demineralised dentine lesions under PH-cycling regime *in vitro*.

Artificial caries lesions were produced then divided into experimental groups: SDF, SDF/KI, FV, and control. The dentine slabs with artificial lesions were subjected to pH cycling regime. Cross sectional Knoop microhardness (KHN) values were taken before and after treatment. The mean difference within groups was compared using Wilcoxon-Signed Rank test. Kruskal-Wallis Test and posthoc pairwise comparison with Bonferroni correction were used to compare KHN between groups.

The KHN of the lesion increases after treatment with SDF, SDF/KI and FV, significant from depths up to 100 μm ($p < 0.05$) whilst no improvement in KHN of W. No significant change in KHN was seen at level 80, 100, and 120 μm for all groups except W. The lesions were softer at deeper levels from 120 to 300 μm for all groups when compared to untreated lesions. The mean difference between W and SDF were highly significant at all levels except at 80 and 100.

The surface morphology analysis under SEM showed clusters of granular spherical grains occluding the dentinal tubules in group SDF and SDF/KI. Group W showed evident demineralisation with exposed tubules. Elemental analysis confirmed the presence of silver particles and fluoride remains on the lesion surface after acidic challenge.

This study showed that SDF has the highest remineralising potential on artificial subsurface dentine lesions after acidic challenge *in vitro*, followed by SDF/KI then FV. SDF also has the ability to minimise lesion progression significantly in the deeper depths compared to other groups, but only confined to the outer dentine lesion. Despite its limitations, the study certainly adds to our understanding of the mechanism of action of SDF in dentine lesions.

Table of contents

Acknowledgement	II
Abstract	III
Table of contents	IV
Lists of Tables	VII
List of Figures	VIII
Abbreviations	XI
Chapter 1 Introduction and Literature Review	1
1.1 Dental caries	2
1.1.1 Composition of enamel and dentine	2
1.1.2 Development of dental caries	3
1.1.3 Saliva role in protecting teeth.....	5
1.2 Fluoride therapy in caries prevention and remineralisation	6
1.2.1 The mechanism by which fluoride controls caries	7
1.2.2 Fluoride reservoir in the oral cavity	8
1.2.3 Toxicity of Fluoride	8
1.2.4 Methods of Fluoride Delivery	11
1.2.5 Fluoride varnishes.....	14
1.2.6 Silver diamine fluoride (SDF)	15
1.3 Model systems used in dental caries research	20
1.3.1 <i>In vitro</i> models for demineralisation and remineralisation	20
1.3.2 Animal caries models	22
1.3.3 <i>In situ</i> models	23
1.4 Demineralisation and remineralisation evaluation techniques	23
1.4.1 Quantitative light induced fluorescence (QLF)	23
1.4.2 Transverse Micro Radiography (TMR)	25
1.4.3 Micro Computed Tomography Scan (Micro-CT Scan)	25
1.4.4 Microhardness test.....	26
1.4.5 Scanning electron microscopy (SEM).....	28
1.5 Summary	28
Chapter 2 Research Aims, Objectives, and Hypothesis	30
2.1 Aim of the study	30
2.2 Objectives of the study	30
2.3 The null hypothesis for the study	30
Chapter 3 Pilot study	31
3.1 Aim of the study:	31

3.2	Materials and Methods:	31
3.2.1	Sample size:.....	31
3.2.2	Tooth selection and cleaning:	31
3.2.3	Preparation of the enamel and dentine slabs:	31
3.2.4	Preparation of the enamel slabs for QLF testing	31
3.2.5	Storage of the enamel and dentine slabs	32
3.2.6	Preparation of the enamel artificial subsurface lesions and dentine artificial subsurface lesions	32
3.2.7	Preparation of the cavitated enamel/ dentine lesions (CEDL).....	33
3.2.8	Experimental groups:	33
3.2.9	Materials:.....	33
3.2.10	Quantitative light-induced fluorescence (QLF) measurements	34
3.2.11	Experimental Protocol/ Regime:	35
3.2.12	QLF images	35
3.3	Statistical Analysis	36
3.4	Results	37
3.5	Discussion	39
3.6	Conclusion	40
3.7	Outcome of the null hypothesis (I)	40
Chapter 4	Materials and Methods	41
4.1	Sample size calculation	41
4.2	Experimental and control groups	41
4.2.1	Materials.....	42
4.3	Dentine slab preparation	44
4.4	Preparation of silicone moulds and resin blocks	47
4.5	Preparation of resin blocks containing dentine slabs	49
4.6	Randomisation and Blinding	50
4.7	Preparation of the dentine artificial subsurface lesion	50
4.7.1	Optimisation of dentine artificial subsurface lesion creation - a pilot study	50
4.8	Cross-Sectional Microhardness (CSMH)	52
4.9	Scanning electron microscopy (SEM)	55
4.10	Experimental protocol/ regime	56
4.10.1	Application of different treatments on dentine lesions surface: SDF, SDF/KI, FV and W	56
4.10.2	The pH cycling regime	58
4.11	Preparations of solutions used in the study	61
4.11.1	Artificial saliva	61
4.11.2	Demineralising solution	62

4.12	Training and calibration	63
4.13	Intra-examiner reliability	63
4.14	Statistical Analysis	63
Chapter 5 Results		64
5.1	Dentine Hardness	64
5.2	Change in dentine hardness within each group.....	66
5.2.1	Change in dentine hardness for SDF group	69
5.2.2	Change in dentine hardness for SDF/KI Group	71
5.2.3	Change in dentine hardness for Group FV	73
5.2.4	Change in dentine hardness for Group W	75
5.3	Difference between groups	78
5.3.1	Difference between levels of indentations between groups	81
5.4	Intra-examiner reliability	84
5.6	Surface Morphology - Scanning Electron Microscopy (SEM)	85
Chapter 6 Discussion.....		91
6.1	In vitro model.....	91
6.2	Study design.....	92
6.3	Dentine slabs preparation and storage.....	92
6.4	Artificial caries lesions.....	93
6.5	pH cycling regime	93
6.6	Cross-sectional microhardness.....	94
6.7	Scanning Electron Microscopy (SEM)	97
6.8	Remineralising potential of Silver Diamine Fluoride (SDF) with or without potassium iodide (SDF/KI) and fluoride varnish (FV) on artificial dentine lesions <i>in vitro</i>	97
6.9	Suggestions for future research.....	103
6.10	Outcome of the null hypothesis (II)	104
Chapter 7 Conclusion		105
References.....		106
Appendix.....		116
Appendix 1- Optimisation of dentine artificial subsurface lesion creation.....		116

Lists of Tables

Table 1-1: Certainly lethal (CLD) and Safety Tolerated Doses (STD) of fluoride for children.....	9
Table 1-2: Common signs and symptoms of acute fluoride toxicity.....	10
Table 1-3: Toxic effects of chronic excessive fluoride ingestion.	10
Table 3-1: Mean values of ΔF at baseline and after treatment for all groups...	37
Table 3-2: Mean values of ΔQ at baseline and after treatment for all groups. ...	38
Table 4-1: Materials used in the study.....	42
Table 4-2: Treatment protocol.....	56
Table 4-3: Day time saliva formulation.....	61
Table 4-4: Night time saliva formulation.....	62
Table 4-5: Demineralising solution constitution 10x stock solution.....	62
Table 5-1: Test of Normality (dentine hardness-KHN)	65
Table 5-2: Change from baseline (Dentine Knoop Hardness – KHN) for SDF and SDF/KI group.....	67
Table 5-3: Change from baseline (Dentine Knoop Hardness – KHN) for FV and W group.	68
Table 5-4: Kruskal-Wallis test for mean difference between four groups.	82
Table 5-5: Pairwise comparison with Bonferroni test for all groups.....	83
Table 5-6: Paired Samples Test for intraexaminer reliability.....	84

List of Figures

Figure 1-1: Schematic representation of remineralisation of the enamel occurring in the presence of fluoride (Featherstone, 1999).....	8
Figure 1-2: A schematic representation of mechanism of action of SDF on tooth with decay (Yamaga et al., 1972).	18
Figure 3-1: Enamel slab under QLF showing the enamel subsurface lesion at baseline.	35
Figure 3-2: Enamel slab after treatment with SDF under QLF showing black discoloration.	35
Figure 3-3: QLF image of enamel subsurface lesion at baseline.....	35
Figure 3-4: QLF image of enamel subsurface lesion after treatment of SDF/KI showing no discolouration.....	35
Figure 3-5: Cavitated enamel/ dentine lesion under QLF at baseline.....	36
Figure 3-6: Cavitated enamel/ dentine lesion after treatment of SDF/KI under QLF.	36
Figure 3-7: Dentine lesion under QLF not.....	36
Figure 3-8: ΔF mean values at baseline and after treatment for all groups.....	37
Figure 3-9: ΔQ mean values at baseline and after treatment for all groups.....	38
Figure 4-1: Silver Diamine Fluoride - SDI Riva Star	43
Figure 4-2: 5% Sodium Fluoride (NaF) varnish - VOCO Proflorid Varnish.....	43
Figure 4-3: Diamond wire saw machine used for the teeth sectioning (Well® Walter EBNER, CH-2400 Le Loche).....	45
Figure 4-4: A whole bovine incisor mounted on a diamond saw using 'green stick' compound (left) and sectioning of enamel from dentine to prepare dentine slabs (right).....	45
Figure 4-5: Individual dentine slab in centrifuge tube.....	46
Figure 4-6: Preparation of dentine slab.....	46
Figures 4-7 a-e: Preparation of silicone mould for resin blocks.	48
Figure 4-8: Custom made silicone mould used for resin blocks.	49
Figure 4-9: Dentine slabs embedded in a resin block with labels.	49
Figure 4-10: Resin blocks immersed in acid gel.....	51
Figure 4-11: Cross-section microhardness of dentine lesion after immersion in demineralisation gel for 3, 4, 5, 6 and 7 days.....	52

Figure 4-12: Cross sectioned resin block containing 5 dentine slabs on a microscope slide.....	53
Figure 4-13: Measuring a vertical distance of indentation from the lesion surface using Duramin 5 software.	54
Figure 4-14: Measuring indent length and Knoop hardness from lesion surface using Duramin 5 software.	54
Figure 4-15: Diagram showing the arrangement of indents on a cross section of a dentine slab.	55
Figure 4-16: Application of SDF onto dentine lesion.	57
Figure 4-17: The patented two-step system SDF (silver capsule) and potassium iodide (green capsule).....	57
Figure 4-18: Application of fluoride varnish on dentine lesion.	58
Figure 4-19: Flow chart of pH cycling regime 28 days.	59
Figure 4-20: Flow-chart of daily pH-cycling regime used in this study.....	60
Figure 5-1: Change in dentine hardness after treatment with Silver Diamine Fluoride (SDF).	69
Figure 5-2: The effect of Silver Diamine Fluoride (SDF) on dentine hardness..	70
Figure 5-3: Change in dentine hardness (KHN) after treatment with Silver Diamine Fluoride + Potassium iodide (SDF/KI).....	71
Figure 5-4: Effect of Silver Diamine Fluoride + Potassium Iodide (SDF/KI) on dentine hardness.	72
Figure 5-5: Change in dentine hardness (KHN) after treatment with Fluoride Varnish (FV).	73
Figure 5-6: Effect of Fluoride varnish (FV) on dentine hardness.	74
Figure 5-7: Change in dentine hardness (KHN) after treatment with deionised water (W).	75
Figure 5-8: Effect of deionised water (W) on dentine hardness.....	76
Figure 5-9: Overall change of dentine hardness (KHN) of the lesions in all groups.....	77
Figure 5-10: Mean difference in dentine hardness between groups.....	79
Figure 5-11: Box-and-whisker plots of mean difference of dentine hardness across groups.	80
Figure 5-12: SEM image: surface morphology of untreated dentine artificial subsurface lesion. 1000x magnification view.	85

Figure 5-13: SEM image: surface morphology of dentine artificial subsurface lesion. 1000x magnification view of dentine artificial subsurface lesion treated with deionised water (W). 86

Figure 5-14: SEM image: surface morphology of dentine artificial subsurface lesion treated with silver diamine fluoride. 100x magnification view. 87

Figure 5-15: SEM image: surface morphology of dentine artificial subsurface lesion treated with silver diamine fluoride. 1000x magnification view. 87

Figure 5-16: SEM image: surface morphology of dentine artificial subsurface lesion treated with SDF followed by potassium iodide. 1000x magnification view..... 88

Figure 5-17: (a) SEM image of surface morphology of artificial lesion treated with SDF 100x magnification; (b) Fluoride elemental mapping of dentine artificial subsurface lesion treated with SDF; (c) Silver elemental mapping of dentine artificial subsurface lesion treated with SDF. 89

Abbreviations

Ag	Silver
AgF	Silver Fluoride
AgNO ₃	Silver Nitrate
Ca	Calcium
CaF ₂	Calcium Fluoride
CEDL	Cavitated Enamel-Dentine Lesion
CSMH	Cross-Sectional Microhardness
DASL	Dentine Artificial Subsurface Lesion
EASL	Enamel Artificial Subsurface Lesion
F	Fluoride
FAp	Fluorapatite
FDA	Food And Drug Administration
FV	Fluoride Varnish
g	Gram(s)
g/L	Gram/ Litre
HCl	Hydrochloric Acid
KHN	Knoop Microhardness
KI	Potassium Iodide
KOH	Potassium Hydroxide
L	Litre(s)
micro-CT	Micro-Computed Tomography
mm	Milimetre
NaF	Sodium Fluoride
NH ₄ OH	Ammonium Hydroxide
PO ₄ ⁻	Phosphate
ppm	Parts Per Million
PTD	Probable Toxic Dose
QLF	Qualitative Light Induced Fluorescence
s	Second(s)
SD	Standard Deviation
SDF	Silver Diamine Fluoride

SDF/KI	Silver Diamine Fluoride/ Potassium Iodide
SEM	Scanning Electron Microscopy
SMH	Surface Microhardness
TMR	Transverse Micro Radiography
W	Deionised Water
w/v	Weight/ Volume
λ	Lambda
μm	Micrometre

Chapter 1 Introduction and Literature Review

Modern dentistry has evolved in ways of treating dental caries since it can cause significant health problems if left untreated. In the past decades, fluoride has been widely used in preventing and reducing the prevalence of dental caries (Petersson and Bratthall, 1996, Petersen and Ogawa, 2016). Despite the advancement of dental technology and extensive dental public health campaigns, the prevalence of dental caries in young children remains high in countries with higher economic growth (El Tantawi et al., 2018). Low exposure to fluoride and free sugar intake are the main factors contributing to the global problem (WHO, 2010, WHO, 2015).

The dynamic process of caries progression and regression are largely affected by fluoride reservoir in the oral cavity. Although arresting active lesion may not be the mainstream treatment option in the world of operative dentistry, application of topical fluorides has helped in reducing caries activity in enamel and dentine (Marinho et al., 2003d, Marinho et al., 2013). Whilst sodium fluoride varnish has been accepted as the 'gold standard' treatment in preventing enamel caries, silver diamine fluoride (SDF) has been proven to effectively arrest caries in dentine (Yu et al., 2018). It is now well established that fluoride plays an important role in preventing and arresting enamel caries. However, the influence of fluoride on dentine has remained unclear. To date, there are only few *in vitro* studies that have investigated the association between the effects of SDF on dentine lesions in comparison to other preventive measures in simulated human oral conditions.

This research examines the emerging role of SDF in the context of its effect on progression or regression on dentine caries lesion after acidic challenge *in vitro*.

1.1 Dental caries

Dental caries is defined as a process that results from localised destruction of susceptible dental hard tissues by acidic by-products from bacterial fermentation of dietary carbohydrates (Longbottom et al., 2009). Caries or carious lesion is defined as "*a detectable change in the tooth structure that results from the biofilm-tooth interactions occurring due to the disease caries*" (Fontana et al., 2010).

Dynamic sequences of microbiological shifts in the plaque biofilm cause dental caries to be a multifactorial, chronic, but reversible disease. Dental caries can be affected by salivary flow and composition, systemic or topical fluoride exposure, the frequency of carbohydrate consumption, and preventative behaviours (Selwitz et al., 2007).

Dental caries is a bacterial based disease caused by cariogenic bacteria such as *Streptococcus mutans* and lactobacilli which are growing as part of the plaque biofilm. These bacteria are acidogenic and therefore have the ability to metabolise fermentable carbohydrates such as glucose, sucrose, fructose or cooked starch into organic acids which cause a pH drop in the plaque biofilm. This will then lead to chemical dissolution and loss of minerals from the enamel, exposed dentine or cementum (Featherstone, 2000).

1.1.1 Composition of enamel and dentine

The enamel and dentine are made up of common constituents. However, they have different structures that will affect the progression of caries and how they react to fluoride.

Permanent enamel is an acellular tissue consists of 85% by volume calcium-deficient carbonated hydroxyapatite minerals that are organised in long and thin apatite crystals forming enamel prisms. The rest of enamel is occupied by water (12% by volume) and organic material (3% by volume) (Buzalaf et al., 2011, ten Cate et al., 2008) where the dynamics of demineralisation and remineralisation take place.

Dentine is the hard portion of the pulp-dentine complex, which is characterised by the presence of multiple closely packed dentinal tubules that extend from the cell bodies of odontoblasts along the entire thickness of the dentine. It consists of 70% inorganic (mineral), 20% organic and 10% water by weight and 45% inorganic (apatite), 33% organic and 22% water by volume (LeGeros, 1991). The carbonated form of hydroxyapatite is the main constituent of the inorganic aspect of dentine. The hydroxyapatite is like enamel but has much smaller dimensions than found in enamel, making the dentine surfaces more susceptible to caries attack. The organic part is mainly composed of collagen (90%) namely Type I collagen with a minor amount of glycoproteins, proteoglycans and phosphoproteins (Buzalaf et al., 2011, Nanci and Ten Cate, 2013).

For many decades and even at the present time, tooth mineral referred to as hydroxyapatite with the following formula:



The apatite crystals in the tooth are contaminated with carbonate and other ions during formation, which makes the crystals more prone to demineralisation than hydroxyapatite. The formula for carbonated apatite can be represented as follows:



The “x”, “y” and “u” level vary slightly throughout the tissue (Featherstone, 2000). Typical levels of impurities in the carbonated apatite are carbonate (4%), sodium (0.6%), magnesium (0.2%) and chloride (0.2%) and minor amounts of fluoride (0.1%) (Driessen and Verbeeck, 1981). Calcium, phosphorus and sodium ion concentration in the dentinal tubules were found to be higher than serum level and increases when caries was formed (Larmas, 1986).

1.1.2 Development of dental caries

The development of dental caries had been extensively researched since the past century. The main characteristics of the caries process are described as follows (LeGeros, 1991, Featherstone, 2004, Deery and Toumba, 2008):

1. Production of organic acids such as lactic, formic, acetic and propionic acid by cariogenic microorganisms when fermentation of carbohydrate occurs in plaque biofilm on the tooth surface;

2. The rapid decrease of pH in the plaque biofilm below the critical pH at which the enamel, dentine or cementum will dissolve when acid diffuses into the underlying tooth structure and minerals such as calcium and phosphate diffuses out from the tooth (demineralisation);
3. The rise in pH within plaque occurs once carbohydrate is unavailable to the plaque microorganisms due to acids diffusing out and calcium and phosphate, together with fluoride diffusing into the tooth, forming a new layer of acid-resistant mineral crystals (remineralisation of enamel) and;
4. This process occurs multiple times throughout the day producing cavitation, repair and reversal, or achieving equilibrium. Dental caries advances only when demineralisation is greater than remineralisation.

The term remineralisation was used to describe “The chemical loss of calcified material from the structure of the tooth, which can be biofilm mediated (caries) or chemically mediated (erosion) from exogenous or endogenous sources of acid (diet, environment, or stomach)” (Longbottom et al., 2009). In the other case, demineralisation was used to describe “The net gain of calcified material within the tooth structure, replacing that which was previously lost through demineralization” (Longbottom et al., 2009).

The dynamic nature of demineralisation and remineralisation in the oral cavity happens several times in a day, with progression or reversal of dental caries being the result. Although the essence of the de- and re-mineralisation processes in dentine are similar to enamel, there are some differences for both substrates. Dentine is more vulnerable to acid attack than enamel because the critical pH of dentine is more than 1 pH unit higher than that for enamel (Buzalaf et al., 2011). A laboratory study comparing the progression of artificial caries lesion in enamel and dentine reported that demineralisation is faster, and remineralisation is slower in dentine when compared to enamel. The porous and larger surface area of dentine crystalline makes it more permeable to acids. Hence, demineralisation of dentine took place at a relatively more considerable depth, when compared to enamel lesions under the same experimental condition (ten Cate et al., 1995). The author concluded that in enamel, mineral uptake and loss occur at similar depths of the lesion. On the other hand, mineral uptake is prevalent at the surface, and mineral loss occurs much deeper in dentine lesion.

Cavitation of the enamel will occur when demineralisation of enamel progresses. However, caries affects dentine prior to the cavitation. Increase in acidity in the enamel stimulates the odontoblasts with possible retraction of the processes and collagen deposition within the periodontoblastic space, which may lead to the deposition of tertiary dentine by the vital odontoblasts. When the collagenous organic matrix is exposed after cavitation of the enamel, the dentine surface is demineralised and destroyed by native and bacterial proteases making the lesion grow larger (Featherstone, 2004). Before this process, acid diffuses ahead of the invading bacteria and demineralises the dentine causing mineral precipitation further into tubules forming more reparative dentine (Nanci and Ten Cate, 2013). The acidic pH in dentine also results in the release of matrix metalloproteinases that causes the destruction of the collagen matrix in dentine (Chaussian et al., 2006). The composition of dentinal tubular fluid also appears to play an important role in lesion formation and progression of demineralisation in dentine (Özok et al., 2004).

1.1.3 Saliva role in protecting teeth

Saliva is an oral fluid that is composed of more than 99% water, and less than 1% solids (mostly electrolytes and proteins), and is produced by the salivary glands at a rate of 0.5-1.0 litres per day (Humphrey and Williamson, 2001, Fejerskov and Kidd, 2008). It is supersaturated with different types of calcium phosphates at the physiological pH of 7.4 (LeGeros, 1991). However, the pH of unstimulated saliva can be as low as 5.6 and increase to 7.8 at very high flow rates (Lagerlöf, 1994). For this reason, human saliva plays an important role in maintaining the integrity of enamel mineral whereby the development, progression and regression of dental caries are believed to be influenced by the degree of saturation of saliva.

Demineralisation occurs in dental tissues as a result of different solubilities of the mineral phases in the dental tissues. Under normal conditions (pH around 7.0), both hydroxyapatite and fluorhydroxyapatite are supersaturated in the oral fluids. Consequently, there is a tendency towards the formation of either calculus or remineralisation of the demineralised dental tissues (Fejerskov and Kidd, 2008).

Cariogenic bacteria produces lactic acid as a result of sugar metabolism in the dental biofilm. During a cariogenic challenge, the lactic acid decreases the pH in saliva and biofilm fluid ($4.5 < \text{pH} < 5.5$), making the fluids undersaturated with respect to hydroxyapatite while still supersaturated with respect to fluorhydroxyapatite, where the hydroxyapatite dissolves from the subsurface and fluorhydroxyapatite forms in the surface layers. Consecutively, the biofilm pH rises within a few minutes due to strong buffering capacity of saliva and outward diffusion of acids. As a result, the supersaturation of hydroxyapatite in the oral fluids will be restored when the pH becomes greater than 5.5, where the demineralised crystals undergo remineralisation (Buzalaf et al., 2011). The supersaturation of oral fluids with respect to fluorapatite during acidic challenges plays an important role in the protection of the surface layer of carious lesions. With time, more fluorhydroxyapatite crystals form after the loss of hydroxyapatite which will result in the concentration of fluorhydroxyapatite in the protective layer and slowing the diffusion of demineralising agents into the lesion (ten Cate et al., 2008).

Bicarbonate, phosphates and other ions are also the essential components in the saliva buffering system. Bicarbonate acts as a buffer by diffusing into plaque and neutralising acids in stimulated saliva. On the other hand, phosphate buffering capacity is more critical in unstimulated saliva (Lagerlöf, 1994). The bicarbonate and phosphate ions help in raising the pH in plaque biofilm after acidic challenge.

1.2 Fluoride therapy in caries prevention and remineralisation

Fluorine is an element that almost never occurs naturally belongs to the halogen group in the periodic table. Due to fluorine's reactivity, it usually exists naturally in fluoride salt form combined with other molecules. Fluoride (F) ion is the simplest anion of fluorine. Fluoride can be found in the Earth's crust mainly as minerals such as fluorapatite, in the gaseous form in the atmosphere and also can be transported by water (WHO, 2010). The concentration of fluoride in the groundwater varies. It depends on the nature of the rock, the water flows through and the occurrence of fluoride bearing mineral in the area. Research has shown that humans obtain approximately 60-80% of fluoride intake from drinking water,

6-8% from cereal products and grains, 5-7% from meat, fish and poultry, and 10-14% from all other foods (Axelsson, 2004).

1.2.1 The mechanism by which fluoride controls caries

For many years, most of the benefit from fluoride was believed to be from systemic intake during tooth development. A multisite, prospective, longitudinal study in Australia revealed that high pre-eruption exposure to fluoride could decrease caries levels significantly in pits and fissures of first permanent molars (Singh and Spencer, 2004). Considering current knowledge, the primary mode of action of fluoride has shown to reduce caries by its topical effects on teeth.

Fluoride inhibits demineralisation by diffusing into plaque and penetrates the crystal surface during an acid challenge. The recently formed crystals structure a type of coating like those of fluorapatite and will not dissolve upon a pH drop caused by acidogenic bacteria, as shown in Figure 1-1. However, in a partially covered surface, uncovered portions of the crystal will dissolve. Fluoride also enhances remineralisation by absorbing into partially demineralised crystals and attracts calcium ions to form calcium fluoride (CaF_2), i.e. a more acid-resistant surface to acidic challenge in the future. However, in dentine, a higher concentration of fluoride is needed to enhance remineralisation and demineralisation when compared to enamel (Buzalaf et al., 2011). Fluoride from topical sources also inhibits essential bacterial enzymes activity in dental plaque (Featherstone, 1999).

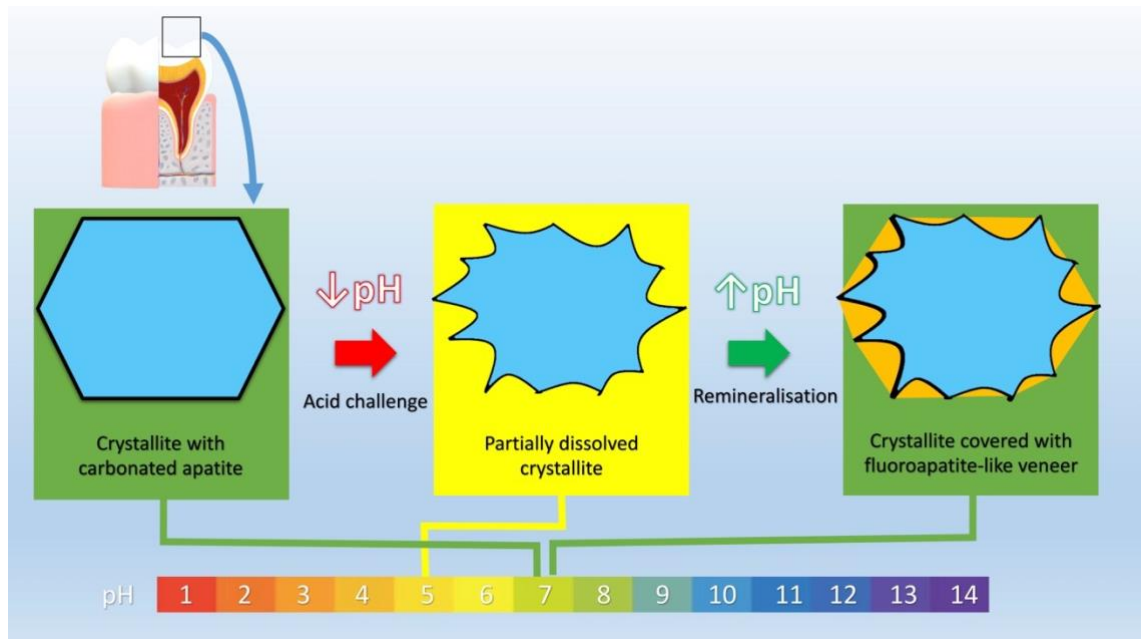


Figure 1-1: Schematic representation of remineralisation of the enamel occurring in the presence of fluoride (Featherstone, 1999).

1.2.2 Fluoride reservoir in the oral cavity

Dental plaque fluids and saliva are the primary reservoirs for fluoride ions in the oral cavity because both are in close proximity with the tooth structure. There are two main types of the oral reservoir for fluoride, which are mineral deposits of fluoride such as calcium fluoride (CaF_2) and fluorapatite (Fap) and biologically or bacterially bound calcium deposits. Although fluorapatite can be found substantially on the tooth surface and caries lesions especially, it is a poor source of oral fluoride and provides little protection to adjacent fluoride-poor tooth minerals. Calcium fluoride in the saliva and plaque liquid becomes supersaturated after application of topical fluoride agents. For this reason, calcium fluoride has long been regarded as the primary source of fluoride in the oral environment (Vogel, 2011)

1.2.3 Toxicity of Fluoride

Fluoride intake is particularly important during tooth development in infants and young children and is responsible in large part for the decline in dental caries globally. The optimum level for fluoride intake is between 0.05 and 0.07 mg F/kg bodyweight for caries prevention effect (Burt, 1992). However, excessive fluoride

ingestion can have a detrimental effect on health (Hodge and Smith, 1965, Jasim et al., 2018, Opinya and Imalingat, 1991).

1.2.3.1 Acute fluoride toxicity

In the past, accidental fluoride poisoning was common, especially in the first half of the 20th century when sodium fluoride was widely used as a pesticide and rat poison. Historically, some fatalities were reported when untreated fluoride ingestion was between 32 -64 mg F/kg bodyweight at a time and Certainly Lethal Dose (CLD) for a 70 kilogram adult was reported between 5 to 10 mg of sodium fluoride (Hodge and Smith, 1965). Safely tolerated dose (STD) for fluoride is a quarter of the CLD (8 to16 mg F per kg), which is the amount that can be ingested without causing acute systemic toxicity. The STDs and CLDs of fluoride for children are given as in table below:

Table 1-1: Certainly lethal (CLD) and Safety Tolerated Doses (STD) of fluoride for children.

Age (yr)	Wt (kg[lb])	CLD (mg)	STD (mg)
2	10.0 (22)	320	80
4	13.2 (29)	422	106
6	16.8 (37)	538	135
8	20.5 (45)	655	164
10	24.1 (53)	771	193
12	29.1 (64)	931	233
14	37.7 (83)	1.206	301
16	41.8 (92)	1.338	334
18	43.2 (95)	1.382	346

Source: (Heifetz and Horowitz, 1986)

Acute toxicity occurs within a short period of time following oral administration or ingestion of excessive fluoride. It first affects the gastro-intestinal system which causes some degree of hyperventilation, abdominal pain, nausea, blood vomiting, diarrhoea followed by general collapse accompanied by pallor, weakness, shallow breathing, weak heart sounds, wet cold skin, cyanosis and equally dilated pupils. In large doses, death may occur within 2-4 hours.

A delayed presentation can be up to 20 hours where paralysis, carpopedal spasm and spasms of extremities occur. This is associated with electrolyte imbalance, particularly severe hypocalcaemia and hyperkalaemia. Serious or life-threatening effect may occur if the total amount of fluoride ingested reaches the Probable Toxic Dose (PTD) of 5.0 mg F/kg of body weight and this should trigger immediate therapeutic intervention of hospitalisation (Whitford, 2011). However, any amount ingested fluoride lower than PTD should not be taken lightly.

The most common signs and symptoms of acute fluoride toxicity are shown in Table 1-2.

Table 1-2: Common signs and symptoms of acute fluoride toxicity.

Low Dosages	High Dosages
Nausea	Convulsions
Vomiting	Cardiac arrhythmias
Hypersalivation	Comatose
Abdominal pain	
Diarrhea	

Source: (Heifetz and Horowitz, 1986)

1.2.3.2 Chronic Fluoride Toxicity

Prolonged exposure to low levels of fluoride can produce chronic fluoride intoxication. Table 1-3 shows the commonly recognised effects of chronic fluoride toxicity.

Table 1-3: Toxic effects of chronic excessive fluoride ingestion.

Effect	Dosage	Duration
Dental fluorosis	>2 times optimal	Until 5 years of age (excluding third molars)
Skeletal fluorosis	10-25 mg/d	10-20 years
Kidney damage	5-10 mg/kg	6-12 months

Source: (Heifetz and Horowitz, 1986)

Long term excessive fluoride can cause dental fluorosis, skeletal fluorosis and kidney damage depending on the age, dosage and duration of exposure (Heifetz and Horowitz, 1986). Dental fluorosis is caused by overexposure to fluoride during the critical period of dental development in young children. It is characterised as mild (a small white opaque appearance on the tooth surface) to hypoplastic, (yellow to brown staining of the enamel) in more severe cases (Dean et al., 1950). Ingestion of high fluoride content in drinking water for a long period of time can also lead to dental and skeletal fluorosis. Two cases were reported in a rural part of Africa involved 11 and 17 year old children presented with dental fluorosis and deformities of upper and lower limbs. In the case report, the area had 10 ppm F in the community drinking water and the reported prevalence of dental fluorosis was high in primary (80%) and permanent (98%) dentition (Opinya and Imalingat, 1991). Chronic fluoride intoxication can also be caused by excess usage of toothpaste due to unusual amount of toothbrushing in adults (Roos et al., 2005). Long term excessive consumption of tea also can lead to various health problems including skeletal fluorosis in adults. However, the systemic effects are reversible once the fluoride intake is controlled. (Jasim et al., 2018).

1.2.4 Methods of Fluoride Delivery

There is a large amount of evidence that fluoride works to reduce caries through many different applications and formulas (Gao et al., 2016, Marinho et al., 2013, Twetman et al., 2003, Marinho et al., 2003d, Marinho et al., 2003b, Marinho et al., 2003c, Marinho et al., 2003a). Besides adding fluoride to drinking water, milk and cooking salt to ensure continuous beneficial fluoride effect can be achieved with little or no individual effort, fluoride is also widely formulated into oral care products.

Traditionally, fluoride delivery methods were generally classified into 'systemic' and 'topical'. However, due to the simultaneous effect of fluoride when ingested and applied topically, a researcher suggested another appropriate classification of fluoride delivery methods as follows (Sampaio and Levy, 2011):

1. Individual methods: fluoridated toothpaste, mouth rinses and supplements.

2. Collective methods: fluoridated water, salt and milk.
3. Professional methods: fluoride varnishes, solutions and foams.

It is also important to note that there is no homeostatic mechanism to maintain the fluoride concentration.

1.2.4.1 Individual methods: fluoride toothpaste, mouth rinses and supplements

Toothpaste or dentifrices were first introduced in the early 1970s. Since their introduction, drastic reductions in caries levels were observed worldwide. Up to now, a number of studies have confirmed the effectiveness of fluoridated toothpaste in caries prevention which has been comprehensively reviewed (Marinho et al., 2003a, Marinho et al., 2003d, Twetman, 2009, Twetman et al., 2003). The evidence for caries prevention property of dentifrices containing fluoride is at a concentration of at least 1,000 ppm F for adult formulations.

A systematic review was conducted to assess the effectiveness and safety of mouth rinse containing fluoride in children and adolescents by (Marinho et al., 2003b). 37 clinical trials were included involving 15813 children and adolescents received supervised fluoride mouth rinses at two main concentrations of sodium fluoride (NaF) daily 230 ppm F or fortnightly 900 ppm F. The review concluded there was a 27% reduction in caries rate in permanent teeth with fluoride mouth rinse compared to placebo or no treatment. There was little information regarding potential adverse effects or acceptability from the trials. A systematic review by Twetman et al. (2004) has concluded similar findings but has highlighted the questionable additional anti-caries effect of fluoride toothpaste used daily by the participants.

Fluoride supplement can be prescribed in tablet, drops, lozenges or chewing gum forms by dental professionals. According to a systematic review, the effectiveness of fluoride supplements in preventing dental caries in primary dentition was inconclusive but reduced the caries rate in permanent teeth (Tubert-Jeannin et al., 2011).

1.2.4.2 Collective methods: fluoridated water, salt and milk.

Water fluoridation is a public health approach to ensure that the recommended optimal fluoride level for dental health reaches the overall population. It involves the controlled adjustment of the fluoride level in communal drinking water. Both the York review (McDonagh et al., 2000) and the Australian National Health and Medical Research Council report (2007) have carried out an extensive review on water fluoridation. They concluded that water fluoridation was effective at reducing caries. There are no other associations of adverse effects that have been confirmed apart from dental fluorosis. A Cochrane review that was conducted in 2015 suggested that more contemporary and controlled research is needed to investigate the effectiveness of water fluoridation for caries prevention (Iheozor-Ejiofor et al., 2015).

Albeit abundant strong evidence showed the anti-caries effect of fluoride through different application and formulation, there is a lack of high quality clinical trials concerning the caries-preventive effect of fluoridated milk, salt and supplement (Espelid, 2009, Yeung et al., 2015).

1.2.4.3 Professional methods: fluoride varnishes, gel and devices.

Many types of professionally applied topical fluoride have been developed and been used in clinical trials or in in vitro studies to arrest caries lesions in enamel and dentine. Cochrane reviews on caries prevention of professionally applied fluoride gels and varnishes showed that they are indeed effective in caries prevention by remineralisation of early enamel caries and arresting dental caries (Marinho et al., 2013, Marinho et al., 2015). In a recent systematic review, the authors concluded that professionally applied 5% sodium fluoride varnish is effective in remineralising early enamel caries and silver diamine fluoride solution at 38% was found to have the capability of arresting active dentine caries (Gao et al., 2016). The relevance of silver diamine fluoride (SDF) for use in preventing caries is clearly supported by current research findings.

Fluoride gels can be applied professionally by dental personnel using high dose fluoride gels (12300 ppm F) in trays or at home by patients using lower dose gels (1000-5000 ppm F). Use of mouth rinses containing 0.2% fluoride and application

of fluoride gels have proved effective in reducing caries rate by 20% in children and adolescent (Poulsen, 2009, Marinho et al., 2015).

Fluoride slow-release devices are a relatively new innovation whereby a small fluoride glass bead is secured to the buccal surface of a molar tooth and continuously releases low levels of fluoride for up to two years. The topic was reviewed by Toumba et al. (2009). However, a recent Cochrane review concluded that the body of evidence available is weak and its applicability to the wider population is still in question Chong et al. (2018). Other means of topical fluoride delivery are fluoride varnishes and silver diamine fluoride which will be discussed in detail below.

1.2.5 Fluoride varnishes

Professionally applied fluoride varnish was developed in the 1960s and has been used as an anti-caries agent in many developed countries around the world. It has been long regarded as the best option in the prevention of dental caries because of the large body evidence for their success in reducing caries activity (Gao et al., 2016, Marinho et al., 2013, Twetman et al., 2003, Marinho et al., 2003d, Marinho et al., 2003b, Marinho et al., 2003c, Marinho et al., 2003a).

The main advantage of fluoride varnishes is the ability to act as slow-releasing reservoirs of fluoride by prolonged contact with tooth surfaces due to its stickiness. Even in a thin layer, the fluoride uptake by tooth tissue increased up to 12 hours or more and with small amounts it can minimise the risk of excessive ingestion (Marinho et al., 2013). The concentration of fluoride is much higher than in gels, with typical concentrations of 22600 ppm F (in sodium fluoride (NaF) varnishes), 7000 ppm F (in difluoro silane varnishes) or 56300 ppm fluoride in (6% NaF + 6% CaF₂ varnishes).

There are many formulations of varnishes available in the market. A large body of evidence supporting the effectiveness of fluoride varnish comes from research using the NaF 22600 ppm F formulation. The most commonly used and studied fluoride varnish products are 5% NaF resin based Duraphat (formulation by Inpharma, Germany), Fluor Protector (difluoro silane formulation by Vivadent,

Liechtenstein). Some of the varnishes available were indicated to treat hypersensitivity but often used off label as caries prevention agent such as Profluorid Varnish (NaF formulation by VOCO, Germany). It contains 10-25% ethanol and 22600 ppm F, and is intended for treating teeth with hypersensitivity and sealing dentinal tubules after cavity preparation. Arends described that fluoride varnishes penetrate into dentine and supposedly 'seals' the dentinal tubules partially or completely to work as root caries prevention and treatment for hypersensitivity (Arends et al., 1997).

Varnishes should be applied two to four times annually to achieve maximum benefits for caries prevention as recommended in many guidelines and reviews. The effectiveness of fluoride has been extensively reviewed by Poulsen (2009) and Pessan et al. (2011) and by the Cochrane review for fluoride varnishes (Marinho et al., 2013). The Cochrane review concluded that there was a moderate quality of evidence in the caries-inhibiting effect of fluoride varnish.

1.2.6 Silver diamine fluoride (SDF)

A concentrated solution of silver nitrate (AgNO_3) has been used for arresting dental caries and for the treatment of hypersensitive dentine for a long time. Various fluorides, e.g., sodium fluoride (NaF) and so forth have been employed for the same purpose. Silver diamine fluoride (38% w/v $\text{Ag}(\text{NH}_3)_2\text{F}$, 30w/w) is a stabilised form of silver fluoride. Silver fluoride (AgF) is the active agent in the solution when it is applied to the tooth surface (Yamaga et al., 1972). This inexpensive material has become established in several countries such as Cuba, Brazil, Japan, Australia and China as a regime for arresting caries and preventing further dental decay in children (Chu and Lo, 2008a, Peng et al., 2012, Rosenblatt et al., 2009). It is available in solution form, is often colourless and comprised of 24.4-28.8% (w/v) silver and 5.0-5.9% fluoride, at a pH 10.0 (Mei et al., 2013a).

1.2.6.1 Role of silver in arresting caries

Silver compounds are known to have antibacterial effects. They have been used as early as the 1840s where silver nitrate (AgNO_3) was used in preventing dental caries in the primary dentition. Silver was then developed for caries prevention in

permanent molars, a cavity cleanser agent and as a dentine desensitiser (Peng et al., 2012). In the 1960s, silver was introduced to be combined with fluoride as an anti-caries agent for a more synergistic effect. However, silver fluoride compounds have limited use in clinical settings because of black staining of the carious lesions (Rosenblatt et al., 2009). The black discolouration of lesions can be avoided through the combined use of silver fluoride and potassium iodide as suggested by (Knight et al., 2006a).

1.2.6.2 Role of potassium iodide in SDF

Potassium iodide (KI) is basically a salt, which is photosensitive and has slightly hygroscopic properties, being highly soluble in water (Martindale, 2009). Available in oral tablets and solution form, it is mainly used to block the uptake of radioactive iodine into the thyroid gland (FDA, 2001), KI and silver are both excellent radiopacifying agents, non-toxic and have strong bactericidal and bacteriostatic effects (Horak et al., 1998).

In dentistry, KI was introduced by Knight et al. (2005) as a new approach to overcome the staining problem caused by the silver ions in SDF. It is applied immediately on the dentine surface after the SDF which will result in a white-creamy precipitate. The precipitate will then be washed away and air dried as recommended by Knight et al. (2006b). The potassium iodide is also effective in minimising the possibility of subsequent staining of an overlying restoration (Knight et al., 2005). However, the salt formed from this reaction, silver iodide, is photosensitive and can turn dark with exposure to light (Zhao et al., 2018).

To date, only a limited number of evidences were found regarding the effectiveness of SDF followed by application of KI (SDF/KI) have been identified. *In vitro* studies by Hamama et al. (2015) and Knight et al. (2007) found that SDF/KI agent is an effective product in reducing the amount of *S. mutans* artificial dentine lesions. Other alternatives have been identified by Zhao et al. (2018) in their review including the usage of ammonium hexafluorosilicate and nano-silver fluoride to minimise the dark staining effect.

1.2.6.3 Mechanism of action of silver diamine fluoride (SDF)

Abundant systematic reviews and studies have shown that the principal action of fluoride is through its topical effect rather than systemic effect (Marinho et al., 2003b, Twetman et al., 2003, Twetman, 2009). A persistent presence of fluoride at low levels (sub-ppm) in the plaque-enamel interface during an acid attack will inhibit demineralisation. When the pH is restored, the traces of fluoride in solution will speed up the remineralisation process (Buzalaf et al., 2011). This indicates that the frequency of application and the constant availability of fluoride is more crucial than the quantity of fluoride administered. The effects of fluoride itself in caries have been widely studied. Nonetheless, the exact role and mechanism of action of silver compounds in SDF are still unclear.

Many *in vitro* studies have been conducted and have shown that the possible mechanism of action of silver might be associated with how it interacts with tooth tissue and its antimicrobial properties against cariogenic bacteria. When SDF is applied topically for treatment of dentine hypersensitivity, it forms a protective squamous layer that plugs the dentinal tubules (Mei et al., 2013b). Therefore, reduced sensitivity is seen in treated patients with hypersensitivity (Castillo et al., 2011, Craig et al., 2012) which is in concordance with the hydrodynamic theory (Markowitz and Pashley, 2008).

Dental caries is a dynamic process involving sugars, bacterial metabolism, demineralisation and organic degradation of tooth structure. A lesion becomes larger when the collagenous organic matrix becomes exposed as a result of demineralisation and destruction of dentine surfaces by bacterial proteases (Featherstone, 2004). A layer of silver-salt forms after SDF is applied on a decayed surface which enhances the demineralised lesion's resistance to acid dissolution and enzymatic digestion (Mei et al., 2012). One of the earliest *in vitro* research studies suggested that SDF reacts with hydroxyapatite to form calcium fluoride (CaF_2), silver phosphate (Ag_3PO_4) and ammonium hydroxide (NH_4OH). CaF_2 then slowly reacts with calcium (Ca^{2+}) and phosphate (PO_4^-) ions in saliva to form insoluble fluorapatites ($\text{Ca}_{10}(\text{PO}_4)_6\text{F}_2$), therefore, increasing acid resistance of the tooth. The chemical reaction that occurs in a tooth with decay after application of SDF is illustrated in Figure 1-2. When SDF reacts with hydroxyapatite, acid resistant fluorapatites was formed, which partly contributes

to its anti-caries effect. Furthermore, CaF_2 occludes the dentinal tubules and prevents the penetration of silver ions and nutrients in bacteria. Significant remineralisation of dentine is found through increased odontoblastic activity (Yamaga et al., 1972). Treated dentine lesions have been shown to have higher mineral density and hardness while the lesion depth decreases (Mei et al., 2013b).

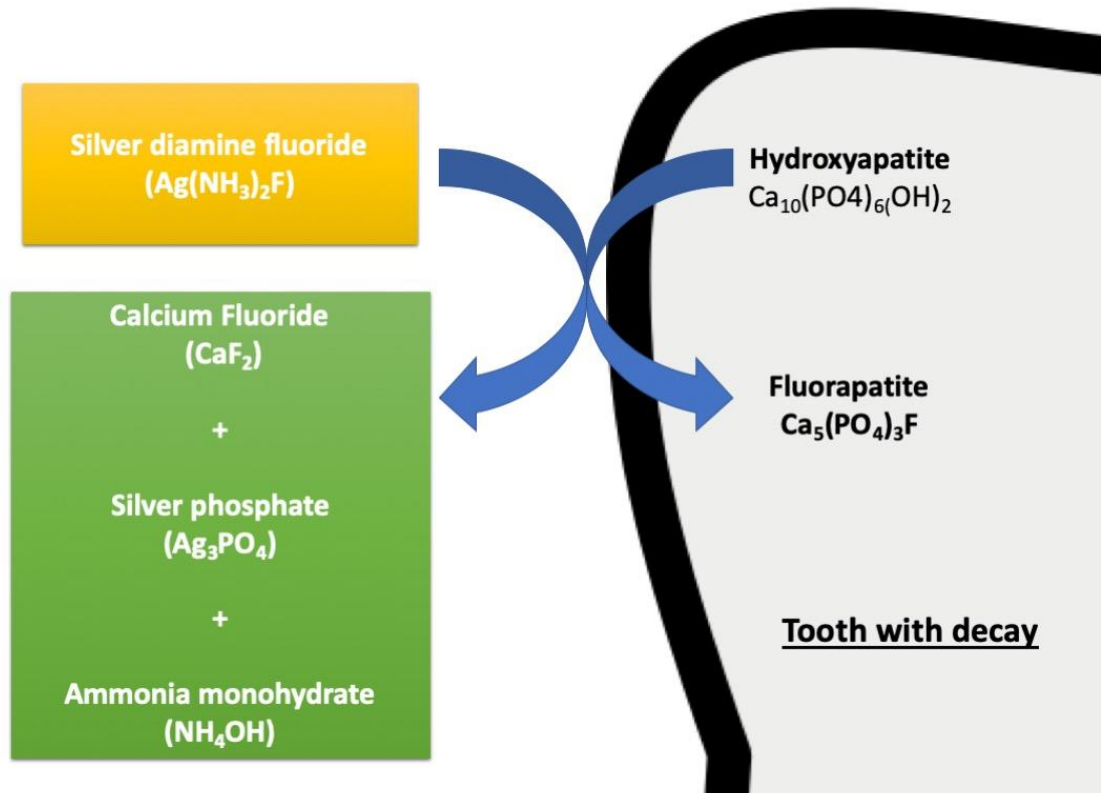


Figure 1-2: A schematic representation of mechanism of action of SDF on tooth with decay (Yamaga et al., 1972).

SDF particularly inhibits matrix metalloproteinases, cathepsins and bacterial collagenases that are responsible for the breakdown of the exposed dentine organic matrix (Horst et al., 2016). Silver in its ionic form reacts directly against bacteria by denaturing enzymes, destruction of the cell wall structure and inhibiting DNA replication (Klasen, 2000, Youravong et al., 2011). *Streptococcus mutans* is highly regarded as the main culprit for dental decay because of its acidogenic properties (Loesche, 1986). Therefore, most research focused on analysing the ability of silver compounds to inhibit *S. mutans*. SDF was proven to perform better than other anti-caries medicaments in inhibiting cariogenic bacteria in dentinal tubules (Hamama et al., 2015).

The mechanism of action of SDF was summarised by Zhao and co-workers in a recently published literature review. This project provided an important opportunity to advance the understanding of how SDF works against dental caries. The review included 29 papers involving *ex vivo* and *in vitro* studies on: (1) the action of SDF on cariogenic bacteria; (2) the effect of SDF on the mineral content of enamel; (3) the effect of SDF on the mineral content of dentine and; (4) the effect of SDF on dentine organic matrix. However, they could not find any clinical trial that studied the mechanism of SDF. A further study with more focus on solving the staining problem of SDF without reducing its effectiveness in arresting dental caries was suggested. The authors concluded that the effectiveness of SDF is mainly due to the bactericidal properties of the silver ion. SDF also possibly arrests caries by inhibiting demineralisation, promoting remineralisation and protecting the collagen matrix from collagen degradation (Zhao et al., 2018).

1.2.6.4 SDF availability in the market

A few types of SDF are available in the market all over the world. In 1972, SDF was accepted to be used as a therapeutic agent by the Central Pharmaceutical Council of the Ministry of Health and Welfare in Japan (Yamaga et al., 1972). August 2014, the Food and Drug Administration (FDA) approved the silver diamine fluoride product for marketing as Advantage Arrest™ by Elevate Oral Care, LLC in the United States (Horst et al., 2016). Riva Star is another fluoride delivery system that has a patented two-step procedure containing SDF (38%) and potassium iodide (KI). It was developed and introduced in Australia for the treatment of dentine hypersensitivity (Knight et al., 2005). Unlike other silver fluoride systems, SDF/KI minimises the risk of black staining, which is a common side effect of topical silver application on carious lesions. By applying the potassium iodide solution over the silver fluoride, a silver iodide creamy white precipitate is formed and giving the 'bleaching' effect. However, very little information is available about the use of SDF in the United Kingdom.

1.3 Model systems used in dental caries research

Caries progression and regression have been widely studied through different approaches. A well conducted, controlled randomised control trial has been regarded as the gold standard in dental caries research. One major drawback of this approach is that it is time consuming and expensive. For this reason, different study models have been developed and adopted in order to mimic the oral environment.

1.3.1 *In vitro* models for demineralisation and remineralisation

Despite the abundance of in situ studies and clinical trials, *in vitro* models are the most frequently used approach in caries research. In this model, tooth specimens either from human or animal were used, and the experiments were done in a laboratory setting by simulation of the oral environment.

In vitro protocols have evolved during the past decades to make studies in dynamics of mineral loss and gain from dental tissues and their reaction to fluoride activity in detail possible (Arends et al., 1989, Arends et al., 1997, Featherstone et al., 1990, Gmur et al., 2006, Hazelrigg et al., 2003, Knight et al., 2007, Knight et al., 2005, Zhao et al., 2017, Zhi et al., 2013). This method is low-cost, yields fast results and is sensitive at the same time. The main advantage of this method is the capability to carry out single variable experiments under highly controlled conditions. However, this method comes with its own limitation; the inability to simulate and replicate precisely the complex biological processes involved in caries and the oral environment (White, 1995).

1.3.1.1 pH-cycling model for artificial lesions

The creation of artificial caries lesions in dentine is an essential tool for the study of the prevention or treatment of dentine lesions. In order to mimic the acid challenge that occurs in the oral environment, the pH cycling model uses demineralisation and remineralisation solutions in the laboratory setting. The model provides alternate phases of demineralisation/remineralisation and consistent changes of the solution prevents saturation (Marquezan et al., 2009).

The model allows a better understanding of the processes and efficacy of fluoride in carious lesions.

The reliability and the caries prevention properties of fluoride containing products by using the *in vitro* pH-cycling model were studied by (Stookey et al., 2011). The authors concluded that the *in vitro* pH cycling model has the capability to measure does response as low as 0 to 1100 ppm F and can statistically separate positive from the negative control. These findings were supported by many other studies that show a dose response to different levels of fluoride in inhibiting demineralisation or remineralisation in human enamel (Rodrigues et al., 2008, Vieira et al., 2005). Therefore, these findings supported that pH cycling is a useful mechanism in assessing the efficacy of fluoride containing materials in control of caries lesions.

1.3.1.2 Source of dental substrates for pH cycling model

Samples originating from human teeth are preferable to be used in *in vitro* and *in situ study* for clinical relevancy. However, there are a few drawbacks when using human teeth. Human teeth are often difficult to obtain in large quantity and good quality since human teeth are often extracted due to caries lesions (Mellberg, 1992b). Human teeth have a larger variation in terms of source, composition, environmental conditions and age. The physical appearance of human teeth is relatively small and the curved surfaces limit the suitability to be used for specific tests that require flat surfaces of uniform thickness (Zero, 1995). Finally, human teeth have been recognised as an infection hazard (Rueggeberg, 1991) and are difficult to obtain due to ethical issues (Skene, 2002). Therefore, other teeth substrates have been suggested to substitute human teeth in dental research.

Bovine teeth have been the most extensively used dental substrate in dental studies. They are easy to source in a large amount, sound without caries, with a more uniform composition compared to human teeth and have relatively large flat surfaces (Mellberg, 1992b). For this reason, specimens obtained from bovine teeth are a suitable alternative to human teeth for *in vitro* pH cycling models. However, a review on bovine teeth as a substitute for human teeth in research concluded that the “morphological, chemical composition and physical property

differences between the two substrates must be considered when interpreting results obtained from any experiment using bovine tooth substrate” (Yassen et al., 2011).

Ideally, human teeth should be used as the substrate in caries research. However, human teeth with good condition are not easy to obtain in large numbers, within a short period of time to create homogenous samples. Furthermore, their use is also restricted by ethical concerns. A considerable amount of evidence has shown bovine dentine is a suitable substrate relative to human dentine instead of from other animal sources (Ortiz-Ruiz et al., 2018, Teruel et al., 2015, Yassen et al., 2011, Zero, 1995, Mellberg, 1992b). Consequently, dentine from bovine teeth were used in the present study. Artificial caries lesions produced from bovine teeth have a mineral distribution and structure that resembles lesions produced from human teeth for both enamel and dentine (Featherstone and Mellberg, 1981, Mellberg, 1992a). An *in situ* study by Hara et al. (2003) suggested that bovine dentine is a suitable substitute for human dentine to evaluate caries development and progression or regression. Bovine dentine is also more porous than human dentine and could lead to ‘over remineralisation’ effect of an artificial dentine caries lesion (Arends et al., 1989).

Despite the similarities between bovine and human dentine, it is important to consider the structure, chemical composition and morphological differences when evaluating the results from a study and translating it to an *in vivo* situation.

1.3.2 Animal caries models

Historically, animal caries models have been a valuable tool in understanding the caries process and the strategies to intervene caries progression including the anticaries effects of fluoride. This model can be controlled by manipulation of the animal’s oral microflora and prescribing a specific diet. Animal models have the advantages over *in vitro* studies as they resemble the caries process in humans very closely.

Rat was the species most used for animal models in testing the effects of topical fluoride products on caries because the caries lesions in rats simulate the human

carries physically, microbiologically and histologically. However, the applicability of animal models was limited as the demineralisation and remineralisation processes do not simulate the human clinical situation. Furthermore, the pH and buffering capacity of animal saliva are dissimilar which restricts the use of this model (White, 1992).

1.3.3 *In situ* models

In situ models are a methodology that utilise a hard-tissue substrate that is mounted on an appliance and placed in the oral cavity in order to analyse the alterations in the substrate due to a treatment or modification of the environment in the mouth. The enamel and dentine are often sourced from human or bovine (Mellberg, 1992b).

The main advantages of *in situ* models are: (1) performed in human oral cavity; (2) more flexible experimental design and could control experimental variables which is difficult in clinical trials; (3) fewer ethical issues; (4) favourable cost factor. However, *in situ* models have generally involved only a small number of subjects, and heavily depend on compliance by the test subjects (Zero, 1995).

1.4 Demineralisation and remineralisation evaluation techniques

1.4.1 Quantitative light induced fluorescence (QLF)

Quantitative light induced fluorescence (QLF) is a sensitive, non-destructive and quantitative method for analysing the changes in mineralisation of enamel specimens that are submitted to *in vitro* or *in situ* experiments (Gmur et al., 2006).

QLF has been developed to detect and monitoring of progression or regression of early caries (Gomez, 2015). The principle of this technique is based on the fluorescence of enamel and the ability of dentine to reflect visible light that has a wavelength (λ) of 370 nm, which is in the blue region of the visible light spectrum. The fluorescence can be observed by using a bandpass filter ($\lambda \geq 540$ nm) that removes the excitation (blue) light, producing images of only green and red

channels and capturing the image with a camera. Loss of fluorescence of the enamel after demineralisation process can be correlated as mineral loss by capturing the image using a camera (intraorally or *in vitro*) and quantifying the results by using a specialised software (Pretty, 2006).

In a hydrated enamel subsurface lesion, an increase in light scattering was seen in comparison to the surrounding enamel. De Josselin et al. (1995) developed a method for assessing the mineral changes in intact and demineralised tooth structure. The currently marketed systems (Inspektor Research Systems BV, Amsterdam, The Netherlands) provide three quantitative metrics:

1. ΔF : Percentage fluorescence loss with respect to the fluorescence of sound tooth tissue; related to lesion depth (%).
2. ΔQ : The ΔF times the Area. Percentage fluorescence loss with respect to the fluorescence of sound tissue times the area. Related to lesion volume (% px²).
3. Area: The surface area of the lesion expressed in pixels² (px²).

Effects of fluoride on remineralisation of enamel lesion have been investigated extensively using QLF technology. In an *ex vivo* study, QLF has been used to detect differences in lesions developed when exposed to an acid challenge and 5% sodium fluoride varnish of different brands applied. The authors found that there was no significant difference ($p > 0.05$) in the amount of remineralisation by the different types of fluoride varnish. They also concluded that QLF was reliable in detecting demineralised and remineralised early carious lesions (Hazelrigg et al., 2003).

However, the use of QLF was limited by the confounding factors that can affect the measurement of fluorescence such as presence of plaque, calculus and staining. No studies were found investigating the effects of silver fluoride and the possibility of its utilisation in detecting dentine lesion using QLF technology. Therefore, one of the aims and objectives of this study is to investigate the feasibility of QLF technology in analysing remineralised artificial caries lesion after application of SDF.

1.4.2 Transverse Micro Radiography (TMR)

The 'gold standard' in determining the mineral loss or gain in artificial caries is transverse micro radiography (TMR) (Buzalaf et al., 2010). This technique assesses mineral content to demonstrate the depth-related properties of carious lesions. Although it is useful in comparing and validating other recently developed caries detection techniques, it is a destructive, time consuming (Lo et al., 2010) and not a reproducible method.

The preparation of the samples for TMR analysis involves producing thin slices of the tooth substrates which is cut perpendicular to the enamel surface. A high resolution micro radiographic image will be obtained by radiographic exposure alongside a calibration aluminium wedge and subsequently compared and calculated for mineral loss or gain (White et al., 1992).

1.4.3 Micro Computed Tomography Scan (Micro-CT Scan)

Micro computed tomography (micro-CT) was first developed around four decades ago and very often used in studying the structure or mineral density of the hard tissues *in vitro* (Lo et al., 2010). This relatively new method for assessment of demineralisation and remineralisation provides precise measurements and very sensitive to changes in mineral with time and position. The micro-CT system produces three-dimensional imaging by using microfocal spot X-ray sources and high-resolution detectors. The images produced represent spatial distribution maps of linear attenuation coefficients determined by the energy of the X-ray source and the atomic composition of the material sample (Swain and Xue, 2009). Through micro-CT, the lesion depth, change in mineral density, morphological and elemental analysis can be analysed in greater detail. It is also non-destructive and reproducible.

A study by Lo et al. (2010) observed the differences between TMR and Micro-CT for studying remineralisation of artificial caries. They concluded that both methods were attested to detect the differences in the artificial caries lesions after a remineralisation process and suggested that micro-CT should be used as a substitute to TMR in *in vitro* studies. These results further support the idea of

using micro-CT as a novel approach in studying tooth demineralisation and remineralisation.

However, the use of micro-CT is limited by the cost of operation and huge amount data processing to reconstruct the three dimensional image; thus, making it difficult and time consuming for data collection in large number of samples (Swain and Xue, 2009).

1.4.4 Microhardness test

This method has been used to determine de- and remineralisation effects on dental enamel (Koulourides and Volker, 1964) and is very sensitive to changes in mineral density (Featherstone and Zero, 1992). Microhardness testing measures the resistance of enamel surfaces to indenter penetration and is a function of the degree of porosity of the superficial enamel layer that indicates mineral loss or gain in subsurface lesions (Koulourides, 1971). This method involved a Knoop or Vickers diamond that is positioned on the sample with a defined load for a given time to create an indentation in the tooth surface. The indentation length is then determined microscopically (in μm) (ten Bosch and Angmar-Mansson, 1991) The microhardness measurements are very sensitive to changes in mineral density and can provide indirect evidence of mineral loss or gain (Featherstone and Zero, 1992).

The following types of microhardness tests exist:

1.4.4.1 Surface Microhardness (SMH)

In this technique, a load with a diamond indenter is applied perpendicular to a polished tissue surface. SMH measurements, when used in the assessment of de/remineralisation, can only give qualitative information on mineral changes, and the samples must have flat surfaces (Arends and Tenbosch, 1992) Furthermore, phenomena such as lesion shape, mineral redistribution, and protein uptake in situ, may affect the indentation length values. A linear relationship between indentation length and lesion depth is valid only in a limited range of lesion depth values (Arends and Schuthof, 1980, Arends et al., 1980, Zero et al., 1990). This technique is non-destructive and allows for a longitudinal

study of the same specimen, however it cannot give details about the subsurface hardness changes or inform about any structural alterations to different sides of the lesion (Featherstone et al., 1983).

1.4.4.2 Cross-Sectional Microhardness (CSMH)

In this technique, the diamond indenter load is applied parallel to the outer anatomical surface (Arends et al., 1980). CSMH experiments provide indirect evidence of mineral change, mineral content and mineral profile (volume % of mineral as a function of distance from the outer surface). The mineral content can be determined quantitatively, and the mineral loss and mineral gain values can be estimated. A study by Magalhaes et al. (2009) aimed to correlate the SMH and CSMH with mineral content, surface layer and lesion depth by TMR in enamel lesions. They concluded that although CSMH can be considered as an alternative to TMR, it does not measure mineral content very reliably. However, CSMH values do provide data about the physical strength of the enamel lesions that TMR is lacking. On the other hand, SMH should not be used to evaluate dental caries lesion as an alternative to TMR.

In dentine lesions, the demineralisation process involves the chemical dissolution of inorganic material and degradation of the organic matrix. (Moron et al., 2013) studied the correlation SMH and CSMH with mineral content, surface layer and lesion depth by TMR in dentine lesions. The authors were in agreement with Magalhaes et al. (2009), where CSMH cannot be used to evaluate mineral content very accurately in dentine lesions. The authors also reported that the hardness measurement at 10 and 30 μm depth from the lesion surface is not reliable due to size and space between the indentations. This approach also is destructive, time consuming and labour demanding, Albeit the limitation of this technique in analysing mineral loss or gain in caries lesions, microhardness remains the only method that take into account the alterations in both organic and inorganic content of dentine (Hara et al., 2003).

Only a small body of literature has studied the mineral content of dentine lesions after treatment with SDF by means of cross-sectional microhardness. A literature review paper showed caries lesions treated with SDF reduces the lesion depth

and had significantly higher surface microhardness, up to 150 μm compared to control lesions treated with distilled water (Zhao et al., 2018).

Chu and Lo (2008a) examined the microhardness of dentine lesions at 30 months after being treated with SDF and sodium fluoride varnish in extracted primary human teeth. Microhardness was determined at every 25 μm from the center of lesions towards pulp up to 500 μm deep. The median of KHN in soft caries lesions was less than 10 KHN and 40 KHN in arrested lesions at level 50 μm of the caries dentine lesion. The KHN value gradually increased as the indentation approaching the pulp and there was no statistical difference between the KHN between soft and arrested dentine caries. This study, however, did not distinguish the difference between the effects of SDF and fluoride varnish and has only 5 samples in each treatment group with no control group.

1.4.5 Scanning electron microscopy (SEM)

The principle of the SEM procedure involves scanning a fine beam of electrons across the surface of a specimen in synchronism with the spot of the display cathode ray tube (CRT). This produces a highly magnified image of three-dimensional appearances, which is derived from the action of the electron beam scanning across the surface of the specimen.

SEM scans qualitative alterations of the surface, the size and the shape of features as well as observing the roughness on the surface of objects. Moreover, SEM can reveal the minerals precipitated in pellicle from mineral dissolution. The advantage of this technique includes no sample preparation is required. The micro-structural images produced by the SEM are readily interpreted. However, SEM only provides subjective and qualitative assessment without comprehensive information about surface alterations of specimens.

1.5 Summary

Despite the importance of SDF, there remains a paucity of evidence on how SDF affects dentine demineralisation. There have been no controlled studies which compare differences in the effects of dentine lesions treated with SDF, SDF with

potassium iodide and sodium fluoride varnish. Therefore, we proposed a study to examine the progression/ regression of enamel and dentine lesions *in vitro* using QLF, Cross-sectional Microhardness and SEM.

Chapter 2 Research Aims, Objectives, and Hypothesis

2.1 Aim of the study

To investigate the effect of Silver Diamine Fluoride (SDF), Silver Diamine Fluoride (SDF) with potassium iodide (SDF/KI) and Fluoride varnish (FV) on progression/regression of artificial subsurface dentine lesions under pH-cycling regime *in vitro*.

2.2 Objectives of the study

- I. To assess the feasibility of Quantitative Light Fluorescence (QLF) to analyse mineral content of artificial enamel and dentine subsurface lesions and cavitated enamel/ dentine lesions treated with SDF and SDF/ KI pilot study *in vitro*.
- II. To investigate the effect of Silver Diamine Fluoride (SDF), SDF with potassium iodide (SDF/KI) and Fluoride varnish (FV) on progression/regression of artificial subsurface dentine lesions under pH-cycling regime *in vitro* using Cross-Sectional Micro Hardness and Scanning Electron Microscopy (SEM).

2.3 The null hypothesis for the study

- I. QLF is feasible in analysing mineral content of artificial enamel and dentine subsurface lesions and cavitated enamel/ dentine lesions treated with SDF and SDF/ KI *in vitro*.
- II. There is no difference in the effect of Silver Diamine Fluoride (SDF), SDF with potassium iodide (SDF/KI) and Fluoride varnish (FV) on progression/regression of dentine artificial subsurface lesions under pH-cycling regime *in vitro*.

Chapter 3 Pilot study

3.1 Aim of the study:

To assess the feasibility of QLF to analyse mineral content of the enamel and dentine artificial subsurface lesion and cavitated enamel-dentine lesions treated with SDF and SDF/KI.

3.2 Materials and Methods:

3.2.1 Sample size:

Convenience sampling of 12 slabs: nine enamel slabs (three per group) and three dentine slabs were used in this study.

3.2.2 Tooth selection and cleaning:

Bovine teeth from a local abattoir were used in this study.

3.2.3 Preparation of the enamel and dentine slabs:

All teeth slabs were prepared from bovine incisors and were stored in distilled water and 0.1% thymol (Sigma Aldrich, thymol 98%) at room temperature. Before the teeth were sectioned, the teeth were cleaned using a spoon excavator and a toothbrush with pumice powder and stone to remove any soft tissue remnants. To detect any defects, caries or cracks, all teeth were screened by transillumination and transmitted light using a low-power microscopy (Leitz, Wetzlar®, Germany).

3.2.4 Preparation of the enamel slabs for QLF testing

Each tooth was then mounted in 'yellow stick' impression compound (Kerr, UK) on plates. After this, the crowns were sectioned using The Well Diamond Wire Saw water cooled, cutting machine (Well@Walter EBNER, CH-2400 Le Loche). For the enamel slabs, the buccal and palatal surfaces of each crown were removed. Then, the enamel slabs were then prepared from these surfaces so that each enamel fragment were approximately 5 mm x 5 mm x 2 mm in sizes.

For the dentine slabs, the root and crown were separated at the cement enamel junction. Mesial and distal surfaces of each crown were removed as well. The dentine slabs were prepared from the buccal surfaces by separating the enamel from the dentine to creating approximately 5mm x 5mm x 2 mm in sizes.

Each enamel and dentine slab were mounted on a plastic rod using “sticky wax” to hold the slab in the demineralising gel. The rod was secured to the lid of a “Sterilin” type universal tube so that when the top was screwed onto the tube, the tooth was suspended in the centre of the tube free space. Two coats of an acid resistant, coloured nail varnish (Max Factor “Glossfinity”) were then applied on the enamel slabs, except for a small window of approximately 2 x 3 mm on the centre of each slab that was left exposed. An interval of 24 hours was left between the two applications to allow the nail varnish to dry completely.

3.2.5 Storage of the enamel and dentine slabs

Once the enamel slabs were prepared, they were kept moist in de-ionised water in “Sterilin” type universal tubes and left at room temperature to prevent dehydration during experiment.

3.2.6 Preparation of the enamel artificial subsurface lesions and dentine artificial subsurface lesions

In order to obtain a sub-surface enamel and dentine lesion an acid demineralising gel was prepared. Preparation of the demineralising system (acidified hydroxyethyl cellulose gel) was performed by adding 0.1 M sodium hydroxide (BDH Analar Grade) to 0.1 M lactic acid (Sigma Aldrich D/L GPR 87% Lactic acid) to give a pH value of 4.5 and then 6% w/v hydroxyethyl cellulose (Sigma Aldrich) was added to the solution and stirred for one hour until a consistency similar to that of “wallpaper paste” was achieved (Issa, 2004). The mixture was left to settle for 24 hours. Once the demineralising gel was ready for use, it was poured into the universal tubes “Sterilin” into which the mounted teeth were then submerged. The enamel and dentine slabs were immersed in acid gel for 10 days to produce an artificial enamel and dentine subsurface lesion. The enamel and dentine slabs

were removed from the acid gel and washed with distilled water; the nail varnish was then removed using methanol to prepare the enamel slabs for the baseline QLF measurements.

3.2.7 Preparation of the cavitated enamel/ dentine lesions (CEDL)

Nail varnish were used to cover the enamel slabs except for a small window of 2mm². The slabs were placed in a demineralisation solution: acidified hydroxyethyl cellulose gel. The gel were prepared by adding 0.1 M sodium hydroxide (BDH Analar Grade) to 0.1 M lactic acid (Sigma Aldrich D/L GPR 87% Lactic acid) to give a pH value of 3.2 and then 6% w/v hydroxyethyl cellulose (Sigma Aldrich) and then added to the solution and stirred for one hour until a consistency similar to that of “wallpaper paste” will be achieved. The mixture was left to settle for 24 hours. Once the demineralising gel is ready for use, it was poured into the universal tubes “Sterilin” into which the mounted teeth will be then submerged. Each tooth slabs were mounted on a spatula. Each slab was immersed in the acid gel for four weeks to produce an artificial cavitated enamel lesion. The slabs were removed from the acid gel and washed with distilled water. The nail varnish was removed using methanol.

3.2.8 Experimental groups:

A Group: Enamel artificial subsurface lesions (EASL) treated with Silver Diamine Fluoride (SDF), (56900 ppm F) applied once.

B Group: Enamel artificial subsurface lesions (EASL) treated with Silver Diamine Fluoride (SDF) (56900 ppm F) with potassium iodide (KI), SDF/KI applied once.

C Group: Cavitated enamel-dentine lesions (CEDL) treated with SDF/KI (56900 ppm F) applied once.

D Group: Dentine artificial subsurface lesions (DASL) with no treatment.

3.2.9 Materials:

1. Silver Diamine Fluoride (SDF), (Riva Star, SDI Ltd, Australia. 56900 ppm F).
2. Silver Diamine Fluoride (SDF) with potassium iodide (KI), SDF/ KI (Riva Star, SDI Ltd, Australia. 56900 ppm F).

3.2.10 Quantitative light-induced fluorescence (QLF) measurements

For each enamel and dentine slab, QLF measurements were taken after the creation of the enamel and dentine subsurface lesion and after the treatment's applications using the QLF machine (QLF-D Biluminator™ 2 Inspektor Research Systems BV, Amsterdam, The Netherlands), under controlled conditions. All the slabs were dried for 15 seconds with compressed air prior to imaging and were then examined in a dark room.

QLF-D Biluminator™ 2 consists of a Biluminator™ mounted on a Single Lens Reflex (SLR) camera fitted with a 60 mm macro lens. The Biluminator™ provides the light sources and filters for making white-light and QLF™-images. Fluorescence images of all enamel specimens were captured with a 'Live View'-enabled digital full-sensor SLR camera (model 550D, Canon, Tokyo, Japan) at the following setting: shutter speed of 1/30 s, aperture value of 6.7, and ISO speed of 1600. All digital images were stored automatically on a personal computer with image-capturing software (C3 version 1.16; Inspektor Research Systems). All fluorescence images were examined with analysing software (QA2 version 1.16; Inspektor Research Systems). The analyses were performed by a single trained examiner.

To ensure that images of the enamel and dentine slabs were always captured in the same camera positions and from the same angles, the camera was attached to a stand in the same position for all the images. The QLF camera was fixed at a position that provided optimum illumination of the enamel block surface. The camera specimen distance was standardised using the jig thereby controlling specimen stability light intensity and magnification.

For each lesion the following metrics will be obtained:

1. ΔF : Percentage fluorescence loss with respect to the fluorescence of sound tooth tissue; related to lesion depth (%).
2. ΔQ : The ΔF times the area. Percentage fluorescence loss with respect to the fluorescence of sound tissue times the area. Related to lesion volume (% px²).

3.2.11 Experimental Protocol/ Regime:

Silver Diamine Fluoride (SDF), Silver Diamine Fluoride with potassium iodide (SDF/KI) were applied topically on the artificial enamel subsurface lesions and artificial cavitated lesions according to manufacturer's instruction. No treatment applied on the dentine artificial subsurface lesion. Then, all groups were analysed with QLF to determine whether silver in SDF and SDF/KI has any effect on the QLF reading (Figure 3-1, Figure 3-2, Figure 3-3, Figure 3-4, Figure 3-5, Figure 3-6, and Figure 3-7).

3.2.12 QLF images

Group A: Enamel artificial subsurface treated with SDF (EASL+SDF)



Figure 3-1: Enamel slab under QLF showing the enamel subsurface lesion at baseline.

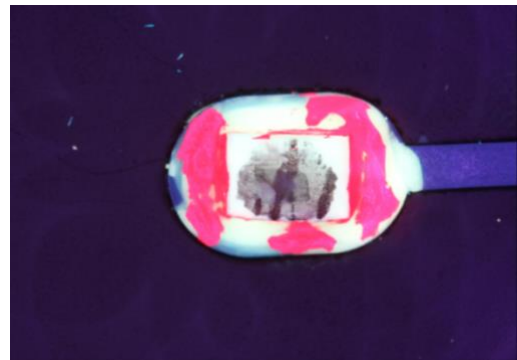


Figure 3-2: Enamel slab after treatment with SDF under QLF showing black discoloration.

Group B: Enamel artificial subsurface lesion treated with SDF/KI (EASL + SDF/KI)

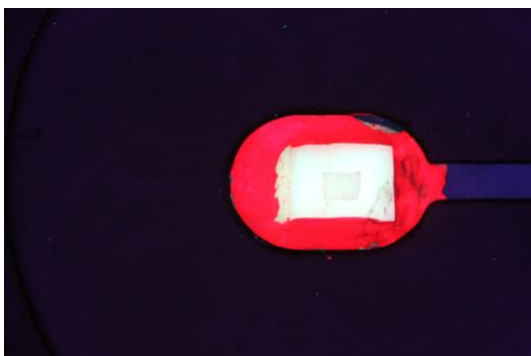


Figure 3-3: QLF image of enamel subsurface lesion at baseline.

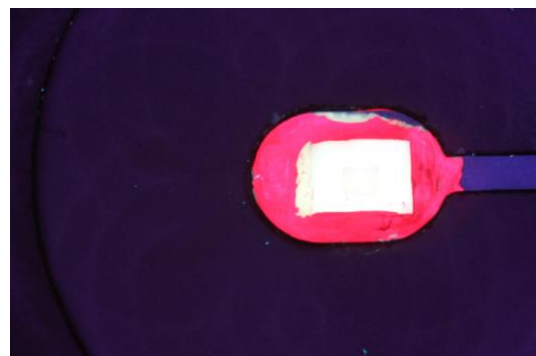


Figure 3-4: QLF image of enamel subsurface lesion after treatment of SDF/KI showing no discoloration.

Group C: Cavitated enamel/ dentine lesion treated with SDF/KI (CEDL+ SDF/KI)

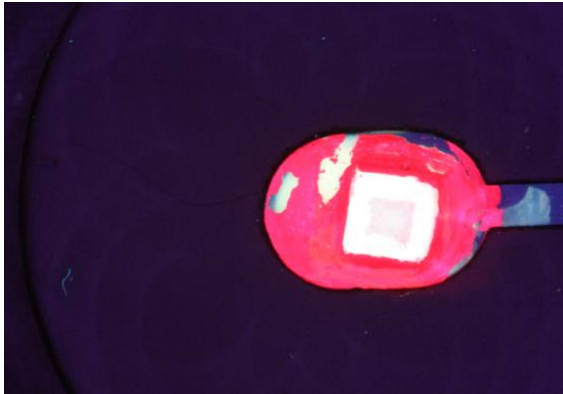


Figure 3-5: Cavitated enamel/ dentine lesion under QLF at baseline.

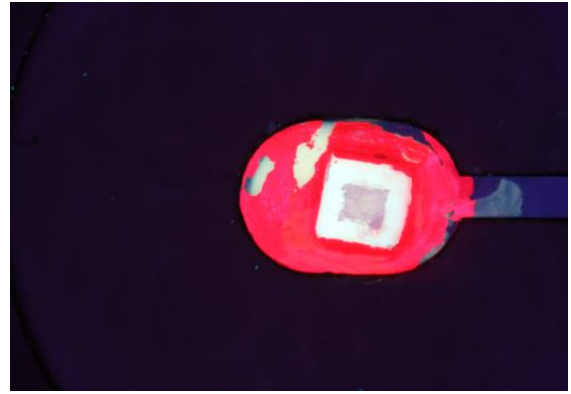


Figure 3-6: Cavitated enamel/ dentine lesion after treatment of SDF/KI under QLF.

Group D: Dentine artificial subsurface lesion (DASL) with no treatment (control)



Figure 3-7: Dentine lesion under QLF not detected.

3.3 Statistical Analysis

SPSS statistical software package (SPSS Inc. ver.22). was used for analysing the data and measure the statistical difference. Descriptive statistics were used to calculate the mean, median, range, and standard deviation. A paired-samples t-test was used to compare the difference between before and after treatment.

3.4 Results

Table 3-1: Mean values of ΔF at baseline and after treatment for all groups.

Groups	Mean ΔF at baseline \pm SD	Mean ΔF after treatment \pm SD	Mean Difference in ΔF at baseline and after treatment \pm SD
EASL+SDF	-20.23 \pm 13.96	-37.10 \pm 17.76	-16.87 \pm 3.8
EASL+SDF/KI	-16.20 \pm 7.44	-13.27 \pm 6.09	2.93 \pm -1.35
CEDL+SDF/KI	-15.03 \pm 7.31	-15.03 \pm 5.12	0 \pm -2.19
DASL	Undetected	Undetected	-

*Paired Samples Test Significance $p < 0.05$

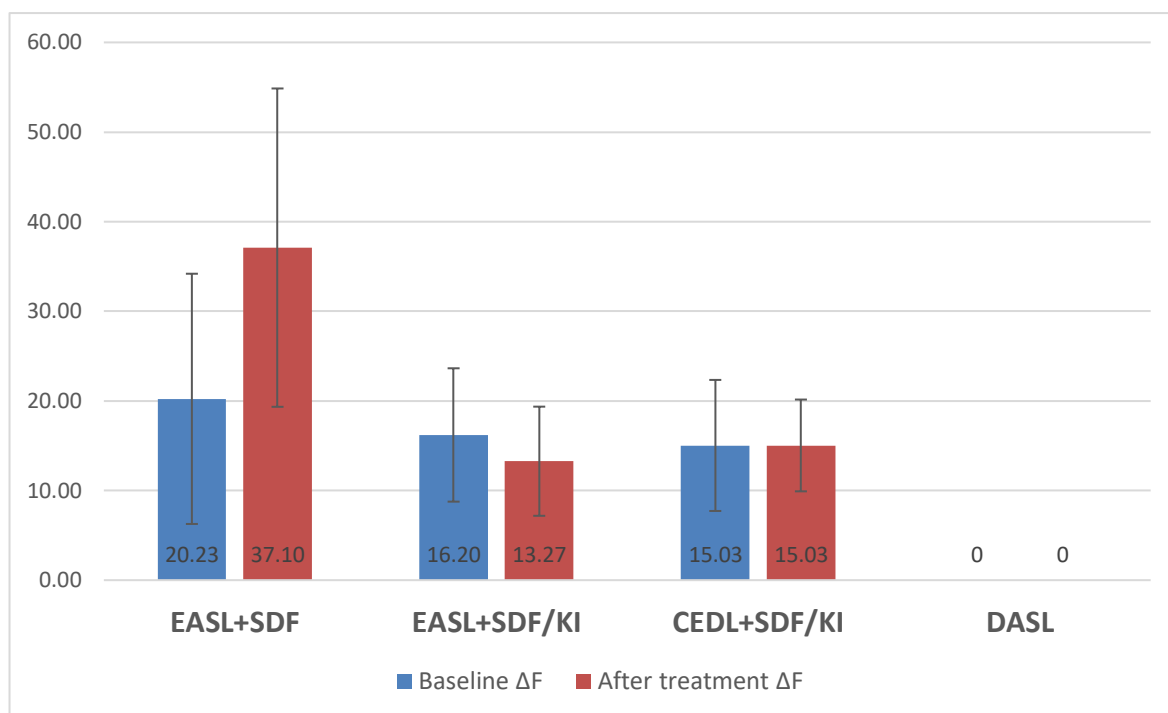


Figure 3-8: ΔF mean values at baseline and after treatment for all groups.

Table 3-1 shows the mean of ΔF at baseline and after treatment and the calculated mean difference of ΔF . The mean difference of ΔF in EASL+SDF group is negative indicating increase in lesion depth (%) whilst other groups showed positive values. In order to assess whether the change in ΔF at baseline and after treatment was significantly different within the same group, paired t-test

was carried out. An increase in lesion depth (ΔF) was seen in EASL+SDF group, however, it is not significant. There was a slight improvement in ΔF values for Group EASL+SDF/KI and almost no change in Group CEDL+SDF/KI. Figure 3-8 shows the change in the mean of ΔF at baseline and after treatment with the error bars representing the standard deviation for all groups.

Table 3-2: Mean values of ΔQ at baseline and after treatment for all groups.

Group	Mean ΔQ at baseline \pm SD	Mean ΔQ after treatment \pm SD	Mean Difference in ΔQ at baseline and after treatment \pm SD
EASL+SDF	-21061 \pm 13828	-63936 \pm 5144	-42875.3 \pm -8683.8*
EASL+SDF/KI	-24865 \pm 18389	-20023 \pm 14094	4842.33 \pm -4294.18
CEDL+SDF/KI	-51207 \pm 27230	-15864 \pm 6461.2	35343 \pm -20769
DASL	-	-	-

*Paired Samples Test Significance $p < 0.05$

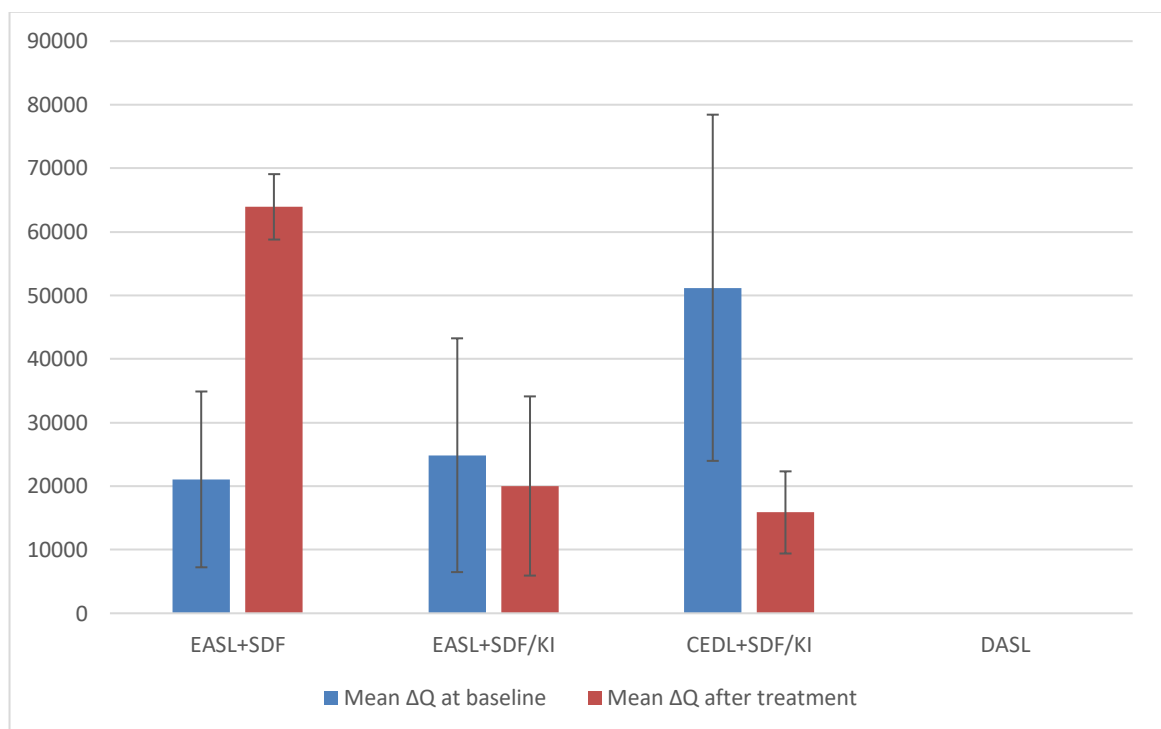


Figure 3-9: ΔQ mean values at baseline and after treatment for all groups.

Table 3-2 shows the mean of ΔQ at baseline and after treatment and the calculated mean difference of ΔQ . The mean difference of ΔQ for EASL+SDF group is negative indicating increase in lesion volume (%) whilst other groups showed positive values. In order to assess whether the change in ΔF at baseline and after treatment was significantly different within the same group, paired t-test was carried out. A significant increase in lesion volume (%) was seen in EASL+SDF group. There was a slight improvement in ΔQ values for Group EASL+SDF/KI and in Group CEDL+SDF/KI. Figure 3-9 shows the change in the mean of ΔQ at baseline and after treatment with the error bars representing the standard deviation for all groups.

3.5 Discussion

QLF is a novel technique for the detection of early demineralisation of enamel. SDF is a colourless solution that forms hardly soluble crystals when in contact with enamel and dentine. However, the solution turns black when it exposed to sun light or reducing agents and forms silver phosphates (Ag_3PO_4) precipitates on the tooth surface (Yamaga et al., 1972).

The present experiment shows a black discolouration of the enamel artificial subsurface lesions appeared almost immediately after the application of SDF. This is a well-known side-effect of silver-based products (Duffin, 2012, Crystal et al., 2017, Horst et al., 2016). The Ag_3PO_4 precipitations formed on the lesion surface might interfere with the detection of the autofluorescence of proteins in dentine. As a result, EASL+SDF group showed a dramatic increase in lesion depth (ΔF) and significantly increase in lesion volume (ΔQ) after treatment with SDF.

On the other hand, the EASL+SDF/KI group showed a decrease in the lesion's depth and volume. However, the reduction was not significant. In group CEDL+SDF/KI, no change was seen in the lesion depth but shown a higher reduction in lesion volume. However, the reduction was not significant. This observation was probably due to the white opaque discolouration produced by KI after application of SDF on the lesion surface, which might affect the autofluorescence of the lesions. SDF followed by KI produced a white-creamy

reaction product called silver iodide (Knight et al., 2005). This step was essential to minimise the discolouration caused by SDF and has been used effectively to treat dentine hypersensitivity (Castillo et al., 2011, Horst et al., 2016, Craig et al., 2012).

In this pilot study, dentine lesion cannot be analysed using QLF due to the high fluorescence of dentine compared to enamel. The lesion in dentine itself produces fluorescence and did not appear dark compared to the surrounding sound tissue. This finding supported by previous studies that showed poor performance of QLF in detecting initial dentine caries in permanent human teeth (Pontes et al., 2017, Jallad et al., 2015, Kuehnisch et al., 2006). There is also no current evidence showing the effectiveness of silver containing products on remineralisation of enamel or dentine caries.

3.6 Conclusion

The evidence from this pilot study suggests that QLF is not a feasible tool in analysing dentine lesions and analysing mineral changes in enamel and dentine lesions treated with silver diamine fluoride, with or without application of potassium iodide. Therefore, QLF was not used in this study to assess the effect of SDF on progression or regression of dentine artificial subsurface lesions.

3.7 Outcome of the null hypothesis (I)

The null hypothesis “QLF is feasible in analysing mineral content of artificial enamel and dentine subsurface lesions and cavitated enamel/ dentine lesions treated with SDF and SDF/ KI *in vitro*” is rejected.

Chapter 4 Materials and Methods

4.1 Sample size calculation

Statistical advice was sought for sample size calculation. The sample size was calculated by using data from a previous study (Mei et al., 2013b). A total sample of 24 dentine slabs per group is needed. This calculation is based on the assumption that the standard deviation of the response variable is 42, and mean difference is 63, power is 84%, 0.05 significance level. This is based on calculations by PASS (Power Analysis for Sample Size) Software. To account for loss, the sample size was increased to 25 dentine slabs per group.

4.2 Experimental and control groups

The dentine slabs with artificial subsurface lesion were randomly assigned according to randomisation table to 4 groups with 25 dentine slabs each as follows:

- **Group 1:** Dentine artificial subsurface lesions (DASL) treated with Silver Diamine Fluoride (**SDF**), applied once.
- **Group 2:** Dentine artificial subsurface lesions (DASL) with Silver Diamine Fluoride with Potassium Iodide, **SDF/ KI** applied once.
- **Group 3:** Dentine artificial subsurface lesions (DASL) treated with 5% Sodium Fluoride varnish (**FV**) applied once.
- **Group 4:** Dentine artificial subsurface lesions (DASL) treated with de-ionised distilled water (**W**) applied once as control.

4.2.1 Materials

Table 4-1: Materials used in the study.

Material	Composition	Manufacturer
Silver Diamine Fluoride (SDF)	Step 1 (Silver capsule) <ul style="list-style-type: none"> • 30–35% silver fluoride • 56900 ppm F • > 60% ammonia solution 	SDI, Bayswater, Australia
Silver Diamine Fluoride + Potassium Iodide (SDF/KI)	Step 1 (Silver capsule) <ul style="list-style-type: none"> • 30–35% silver fluoride • 56900 ppm F • > 60% ammonia solution Step 2 (Green Capsule) <ul style="list-style-type: none"> • Saturated KI solution 	SDI, Bayswater, Australia
VOCO Proflorid Varnish (FV)	<ul style="list-style-type: none"> • 5% Sodium Fluoride • 22600 ppm F 	Voco GmbH, Germany.
Deionised water (W)	Deionised water	



Figure 4-1: Silver Diamine Fluoride - SDI Riva Star



Figure 4-2: 5% Sodium Fluoride (NaF) varnish - VOCO Profluorid Varnish

4.3 Dentine slab preparation

All teeth slabs used in this study were obtained from bovine incisors and were stored in distilled water and 0.1% thymol (Sigma Aldrich, thymol 98%) at room temperature. Before the teeth were sectioned, the teeth were cleaned using a spoon excavator and a toothbrush with pumice powder and stone to remove any soft tissue remnants. To detect any defects, caries or cracks, all teeth were screened by trans-illumination and transmitted light using a low-power microscopy (Leitz, Wetzlar®, Germany). Suitable teeth were selected to be used for this present study.

The cleaned teeth were dried and attached whole with 'green stick' impression compound (Kerr, UK) on plates that fitted into the cutting machine 'The Well Diamond Wire Saw, water-cooled, cutting Machine' (Figure 4-3). The crown was sectioned using a water cooled, diamond wire saw, cutting machine (Well@Walter EBNER, CH-2400 Le Loche). The root and crown were separated at the cement enamel junction as shown in Figure 4-4. Mesial and distal surfaces of each crown were removed as well. The dentine slabs were prepared from these surfaces by separating the enamel from the dentine to creating 2 mm thick slabs.

Finally, the dentine slabs were separated into small sections so that each dental fragment will be approximately 6mm x 3mm x 2mm in size. Each sample was randomised into treatment groups, numbered and stored individually in a centrifuge tube (Figure 4-5). Each specimen was sectioned again to create a pair of control group with size approximately 3mm x 3mm x 2mm.

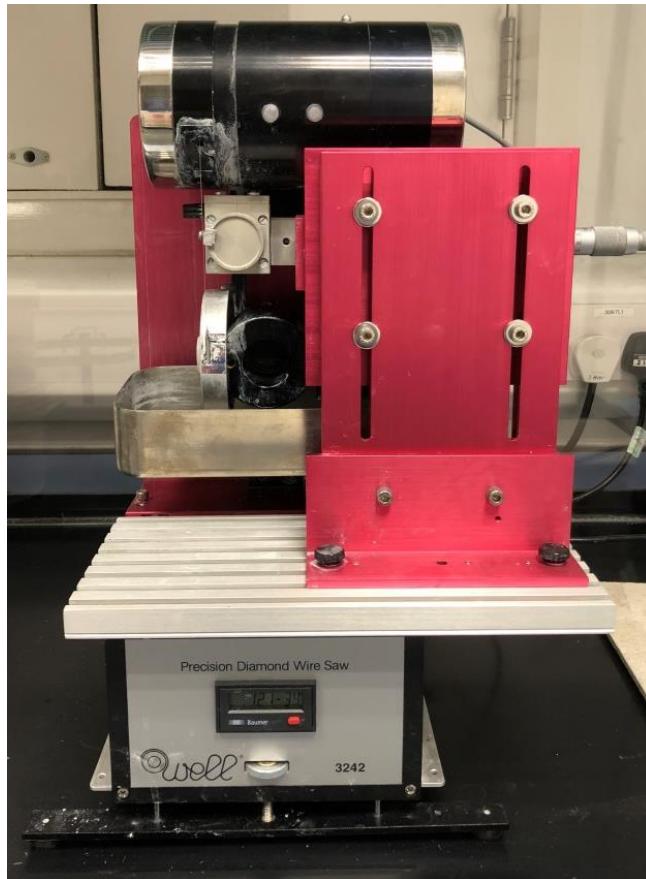


Figure 4-3: Diamond wire saw machine used for the teeth sectioning (Well® Walter EBNER, CH-2400 Le Loche).

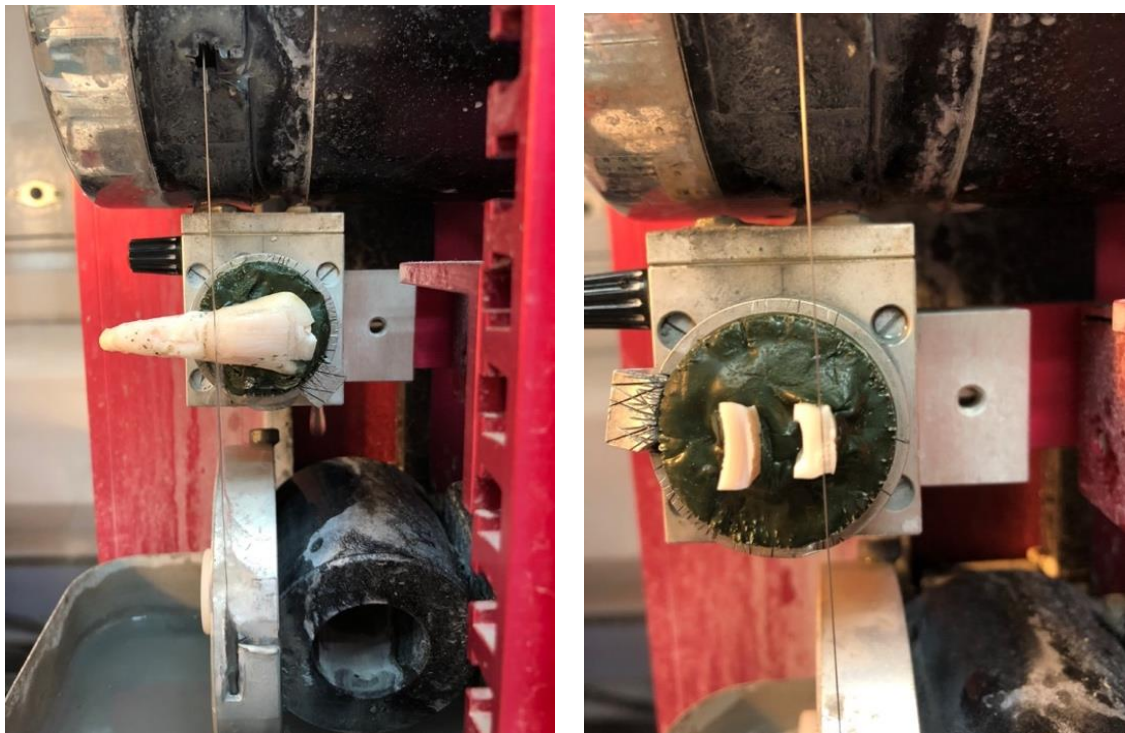


Figure 4-4: A whole bovine incisor mounted on a diamond saw using 'green stick' compound (left) and sectioning of enamel from dentine to prepare dentine slabs (right).



Figure 4-5: Individual dentine slab in centrifuge tube.

a) Dentine slab after separated from enamel were cut into a pair

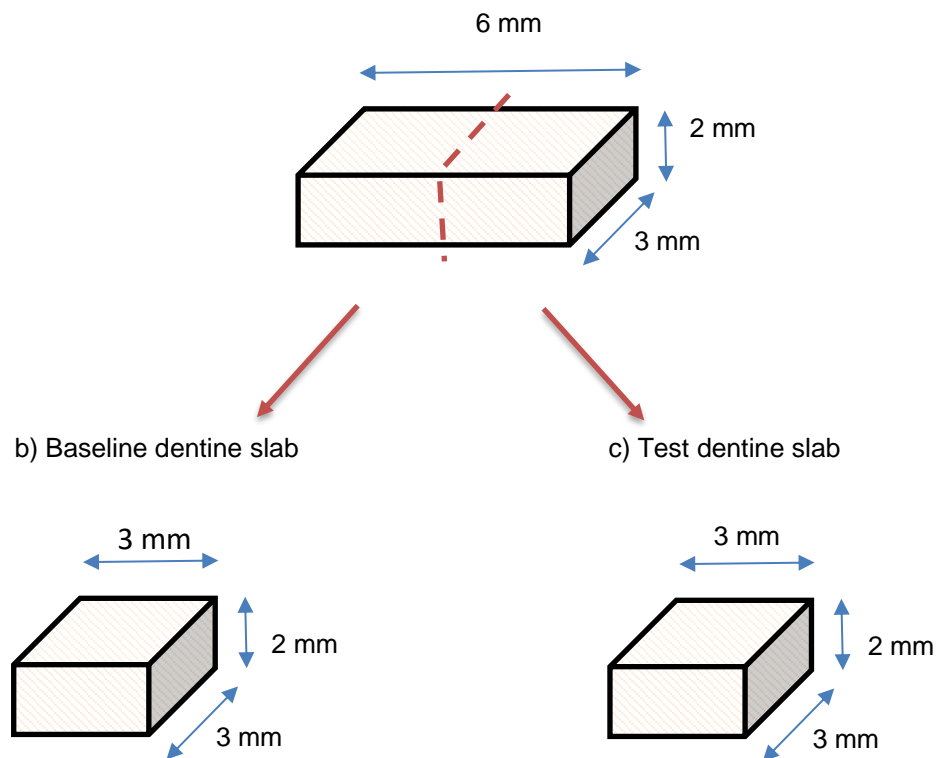
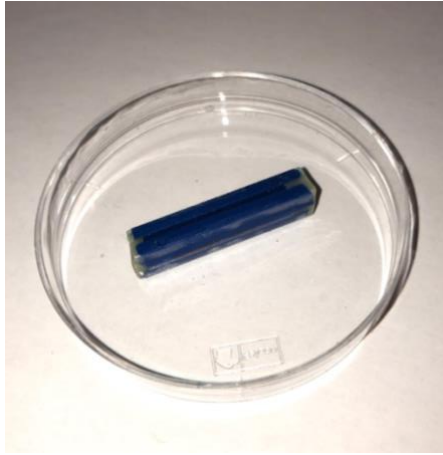


Figure 4-6: Preparation of dentine slab.

4.4 Preparation of silicone moulds and resin blocks

A block of 6 mm x 6 mm x 30mm was made from rectangular plastic rods to creating a cast for the resin blocks. They were secured with Loctite® Super Glue to the base of a petri dish in order to create a cast for the silicone mould. A high strength silicone mould making rubber (SILASTIC® S Base/Curing Agent) was mixed 9:1 and poured into the cast and left to set overnight. The silicone mould was removed from the cast and inspected for defects. Clear resin (Stycast 1266: Hitek Electronic materials, Scunthrope, UK) was mixed 3:1 and pipetted into the silicone mould to create 3 resin blocks and left to set for 24 hours. The blocks were glued to the base of a new petri dish and then a new silicone was made (Figures 4-7 a-e).



a) Plastic rectangular block glued to base of a petri dish.



b) Silicone mould for single resin block.



c) Resin blocks created from silicone mould.



d) Resin blocks glued to base of a petri dish.



e) Silicone mould ready for preparation of resin blocks containing dentine slabs.

Figures 4-7 a-e: Preparation of silicone mould for resin blocks.

4.5 Preparation of resin blocks containing dentine slabs

The slabs were placed in silicone moulds, embedded in clear resin with its appropriate labels and left for 24 hours to dry in order to create a block of 6 mm x 6 mm x 30mm. Each resin block contains five dentine slabs. The surface of the dentine slabs was exposed and polished with 800, 1200, and 2000-grit sandpapers. Once the dentine slabs were prepared, they were kept moist in custom made tubes containing distilled water at room temperature to prevent dehydration.

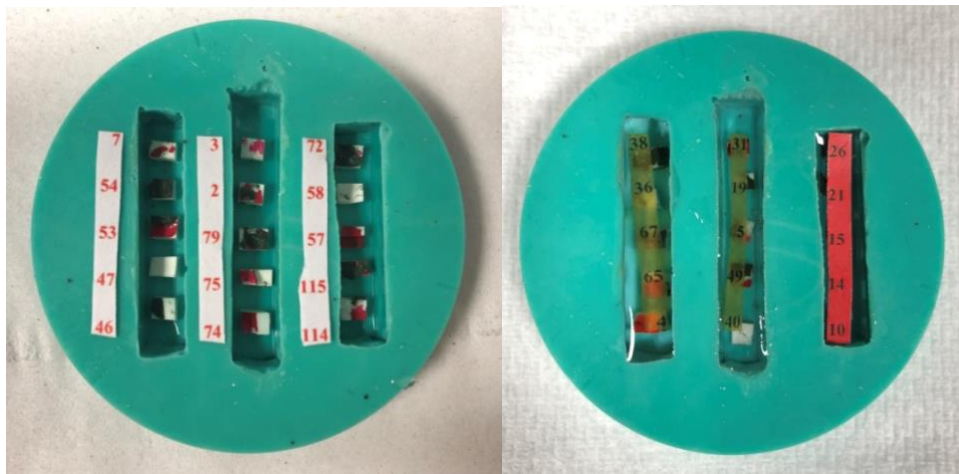


Figure 4-8: Custom made silicone mould used for resin blocks.

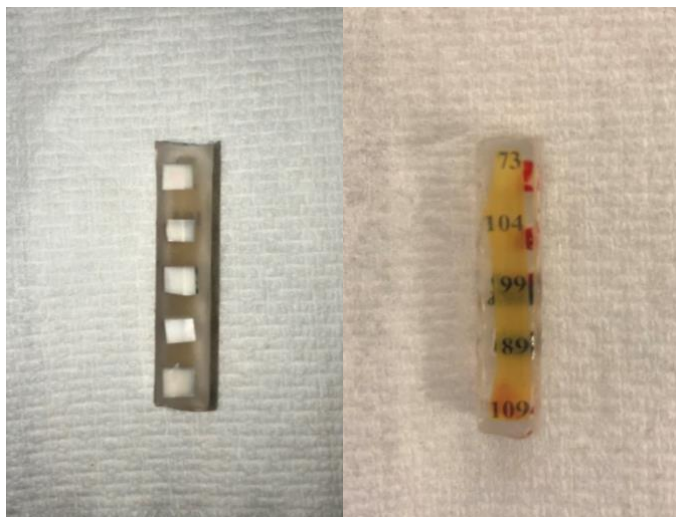


Figure 4-9: Dentine slabs embedded in a resin block with labels.

4.6 Randomisation and Blinding

Prior to mounting the dentine slabs in the resin blocks, they were randomly assigned to four experimental groups using a random number generator and relabelled. The codes were kept with another member of staff. White label with red fonts were used for the 'baseline' dentine slabs and colour-coded labels with black fonts were used for the 'test' dentine slabs. The study investigator, who carried out the testing, were blinded to the codes of tooth slabs during the baseline reading. However, the dentine slab that undergone the pH cycling regime could not be blinded due to different methods of the application of each treatment group.

4.7 Preparation of the dentine artificial subsurface lesion

A pilot study was carried out in order to determine the appropriate period of demineralisation to produce a consistent lesion of at least 100µm depth on each dentine sample.

4.7.1 Optimisation of dentine artificial subsurface lesion creation - a pilot study

21 bovine dentine slabs were used in this pilot study. The dentine slabs in resin blocks were placed in a demineralisation solution: acidified hydroxyethyl cellulose gel. The gel was prepared by adding 0.1 M sodium hydroxide (BDH Analar Grade) to 0.1 M lactic acid (Sigma Aldrich D/L GPR 87% Lactic acid) to give a pH value of 5 and then 6% w/v hydroxyethyl cellulose (Sigma Aldrich) were added to the solution and stirred for one hour until a consistency similar to that of "wallpaper paste" achieved. The mixture was left to settle for 24 hours. Once the demineralising gel is ready for use, it was poured into the custom-made tubes where the resin blocks containing dentine slabs were then submerged. Each slab was immersed in the acid gel for maximum of seven days to produce an dentine artificial subsurface lesion Figure 4-10.



Figure 4-10: Resin blocks immersed in acid gel.

Three dentine slabs with artificial lesion in the resin blocks were removed from the acid gel on the day 3rd, 4th, 5th, 6th, and 7th and washed with distilled water. The resin blocks with dentine artificial subsurface lesions were sectioned through the centre, exposing the subsurface lesion. The cross-sectioned surface of the dentine artificial subsurface lesions was serially polished with 1200, and 2000-grit sandpapers. The specimens were washed and stored in deionized water.

The mineral content of dentine artificial subsurface lesions was analysed by using cross-sectional microhardness. Values of Knoop hardness were obtained from the lesion surface every 20 μm at thirteen levels of depth (20, 40, 60, 80, 100, 120, 140, 160, 180, 200, 220, 240, 260 μm), by using a static load of 10 g applied for 5 seconds, in order to find and record the values of intact dentine hardness. Three sets of indentation were made 100 μm apart.

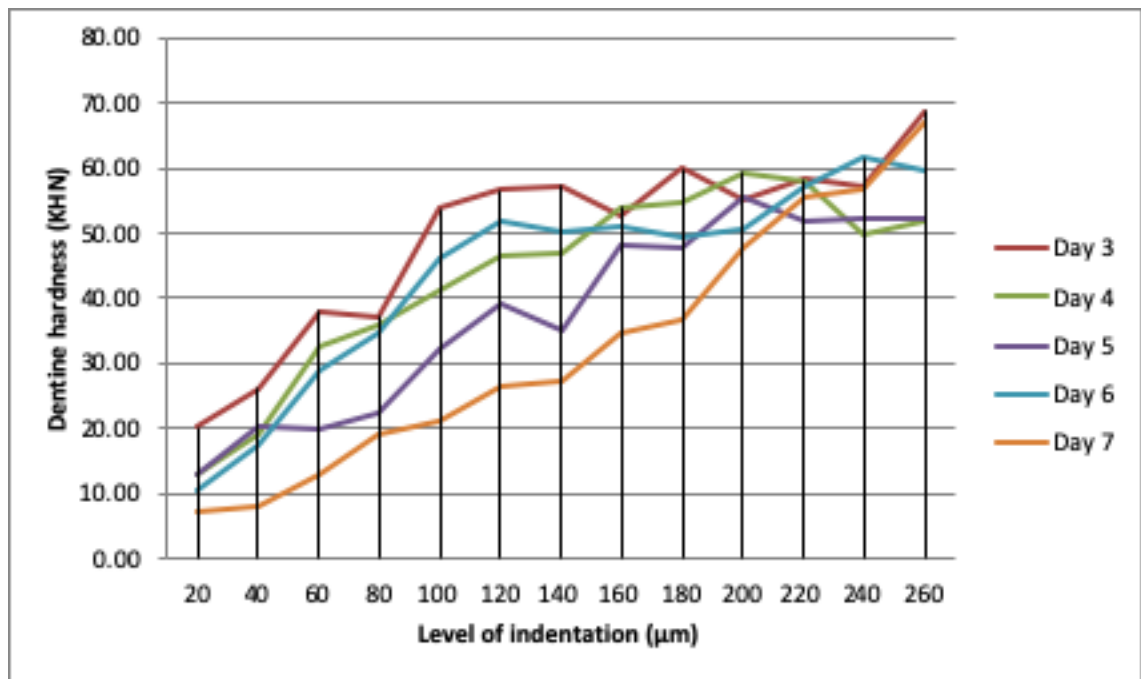


Figure 4-11: Cross-section microhardness of dentine lesion after immersion in demineralisation gel for 3, 4, 5, 6 and 7 days.

The graph shows a trend of increasing Knoop microhardness value and concentrates at the value between 50-70 KHN (intact dentine hardness) starting at 200 µm depth (Appendix 1). The duration for dentine slabs immersion in demineralising gel is determined at five days, equivalent to 120 hours to create lesion no less than 180 µm.

In order to ensure the uniform lesion depth created for the main study, 100 dentine slabs were immersed in freshly prepared acid gel for five days (120 hours). The slabs were removed from the acid gel, washed with distilled water and stored in distilled water at all times.

Consistent hardness of the dentine lesions was established from this protocol. Therefore, cross sectional microhardness was used as the main method of analysis for this study.

4.8 Cross-Sectional Microhardness (CSMH)

One hundred dentine slabs were used in this main study. For each dentine slab, the mineral content of dentine artificial subsurface lesions was analysed at baseline and after pH cycling regime using Cross-sectional Microhardness

(CSMH). The resin blocks containing dentine slabs with artificial lesion will be sectioned through the centre, exposing the subsurface lesion. The cross-sectioned surface of the artificial lesion will be serially polished with 800, 1200, and 2000-grit sandpapers. Each resin block were then securely mounted on a piece of microscope slab using Loctite® Super Glue and was labelled appropriately as shown in Figure 4-12. CSMH were assessed using a computer-aided Duramin Indenter Machine (Struers A/S, DK 26-10, Denmark) and vertical distance measured (Figure 4-13). Each indentation was made using a Knoop diamond under a 10g load for 5 seconds. Indentations were made on the cross-sectioned surface of the dentine artificial subsurface lesion. Measurements of Knoop hardness (KHN) and indent length (μm) were obtained perpendicularly from the lesion surface every 20 micrometre at fifteen levels of depth (20, 40, 60, 80, 100, 120, 140, 160, 180, 200, 220, 240, 260, 280 and 300 μm) and were repeated three times (Figure 4-15).



Figure 4-12: Cross sectioned resin block containing 5 dentine slabs on a microscope slide.

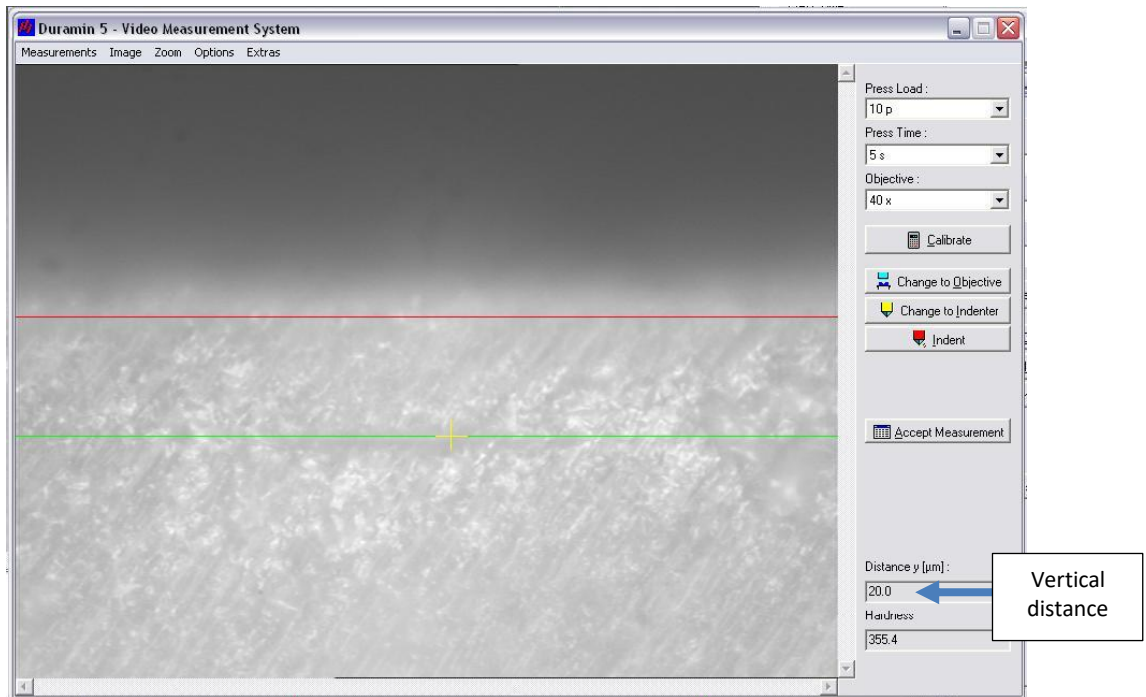


Figure 4-13: Measuring a vertical distance of indentation from the lesion surface using Duramin 5 software.

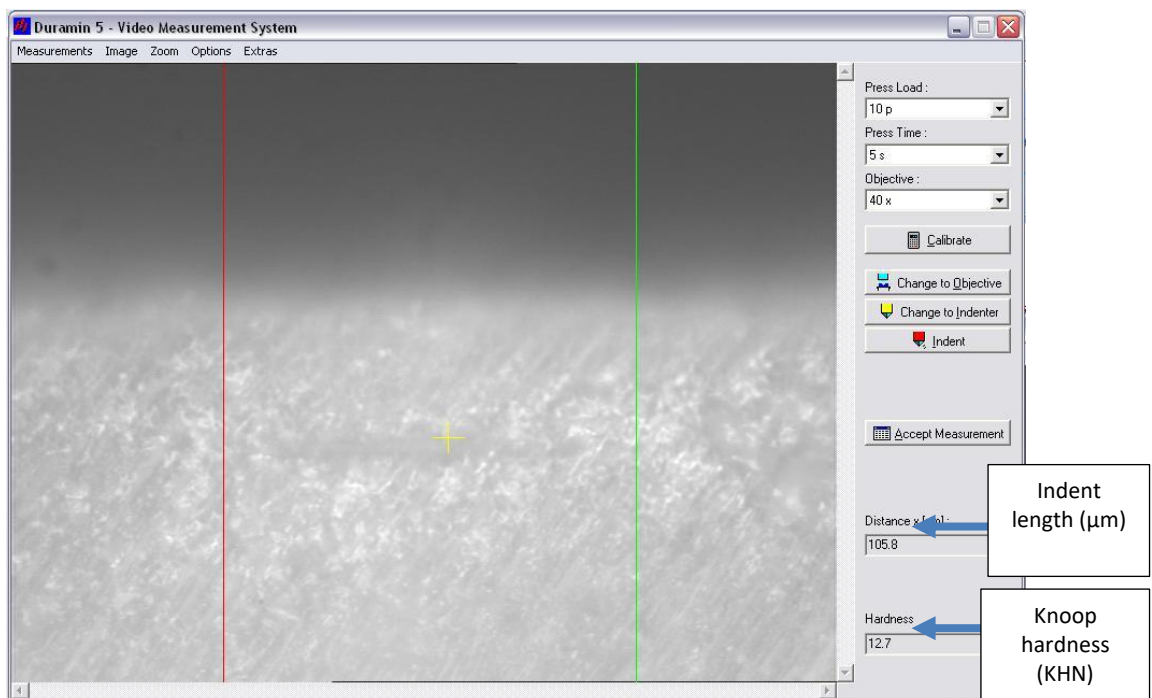


Figure 4-14: Measuring indent length and Knoop hardness from lesion surface using Duramin 5 software.

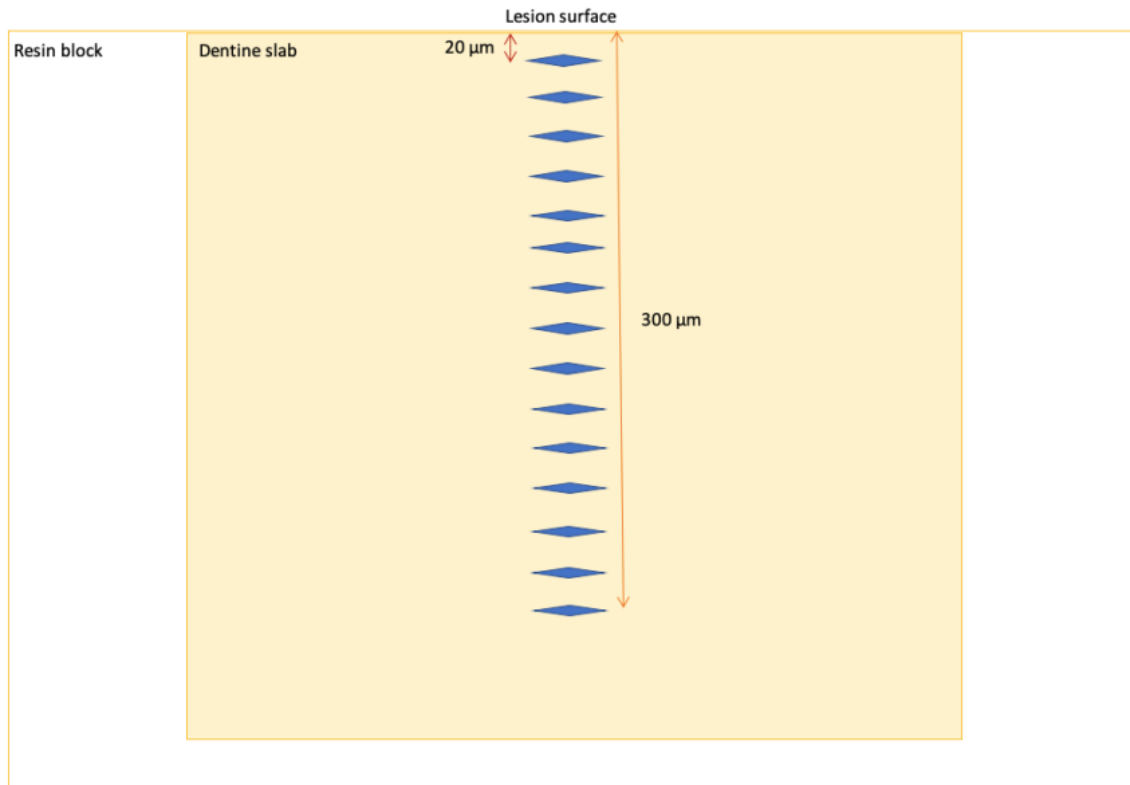


Figure 4-15: Diagram showing the arrangement of indents on a cross section of a dentine slab.

4.9 Scanning electron microscopy (SEM)

Dentine samples were dried in a desiccator and sputter-coated with gold. The microstructural analysis of the specimens was examined under scanning electron microscope (HITACHI S-3400N) operated at a 20-kV accelerating voltage. Elemental analysis and mapping were carried out using EDAX to assess the silver and fluoride on the dentine surface.

4.10 Experimental protocol/ regime

4.10.1 Application of different treatments on dentine lesions surface: SDF, SDF/KI, FV and W

The experimental materials were applied on the dry dentine lesion surface (Table 4-2).

Table 4-2: Treatment protocol

Group	Protocol
Silver diamine fluoride (SDF)	The silver brush (included in the kit) was used to pierce the protective coil of the silver capsule then dipped in the solution and applied on the lesion surface for 1 minute.
Silver diamine fluoride + potassium iodide (SDF/KI)	The silver brush (included in the kit) was used to pierce the protective coil of the silver capsule then dipped in the solution and applied on the lesion surface. Then, the green brush is used to pierce the foil of the green capsule. A generous amount of solution from green capsule applied on the lesion surface until creamy white turns clear for 1 minute.
Fluoride varnish (FV)	A thin layer of varnish applied on the lesion surface using a brush for 1 minute.
Distilled water (W)	A thin layer of distilled water applied on the lesion surface using a brush for 1 minute.

After application of each material, the dentine artificial subsurface lesions were left to air dry for one hour and stored in distilled water to avoid dehydration.



Figure 4-16: Application of SDF onto dentine lesion.



Figure 4-17: The patented two-step system SDF (silver capsule) and potassium iodide (green capsule).

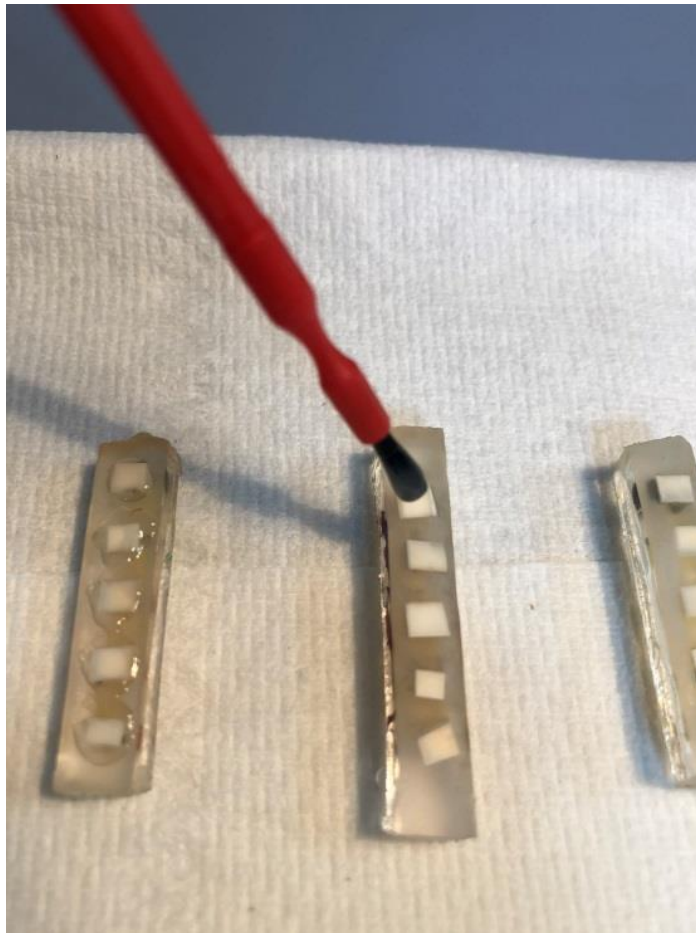


Figure 4-18: Application of fluoride varnish on dentine lesion.

4.10.2 The pH cycling regime

All groups of dentine slabs with artificial lesion were subjected to a pH cycling regime. The dentine slabs with artificial lesion were rinsed with distilled water and immersed in 150 ml of demineralisation solution 5.0 mM Acetic Acid pH 4.8 for 5 minutes. Then, they were rinsed with distilled water for 1 minute and suspended in 150 ml of day time saliva for a minimum of 60 minutes. This procedure was repeated 5 times per day. After the fifth dipping in the demineralisation solution the slabs were rinsed with distilled water for 1 minute and immersed into day time saliva pH 6.8 and kept in the night time saliva pH 6.8 overnight. The dentine slabs with artificial lesion were kept in an incubator at 37°C at all times except when being immersed into treatment solutions. Duration of the pH cycling regime was 28 days (Figure 4-19 and Figure 4-20).

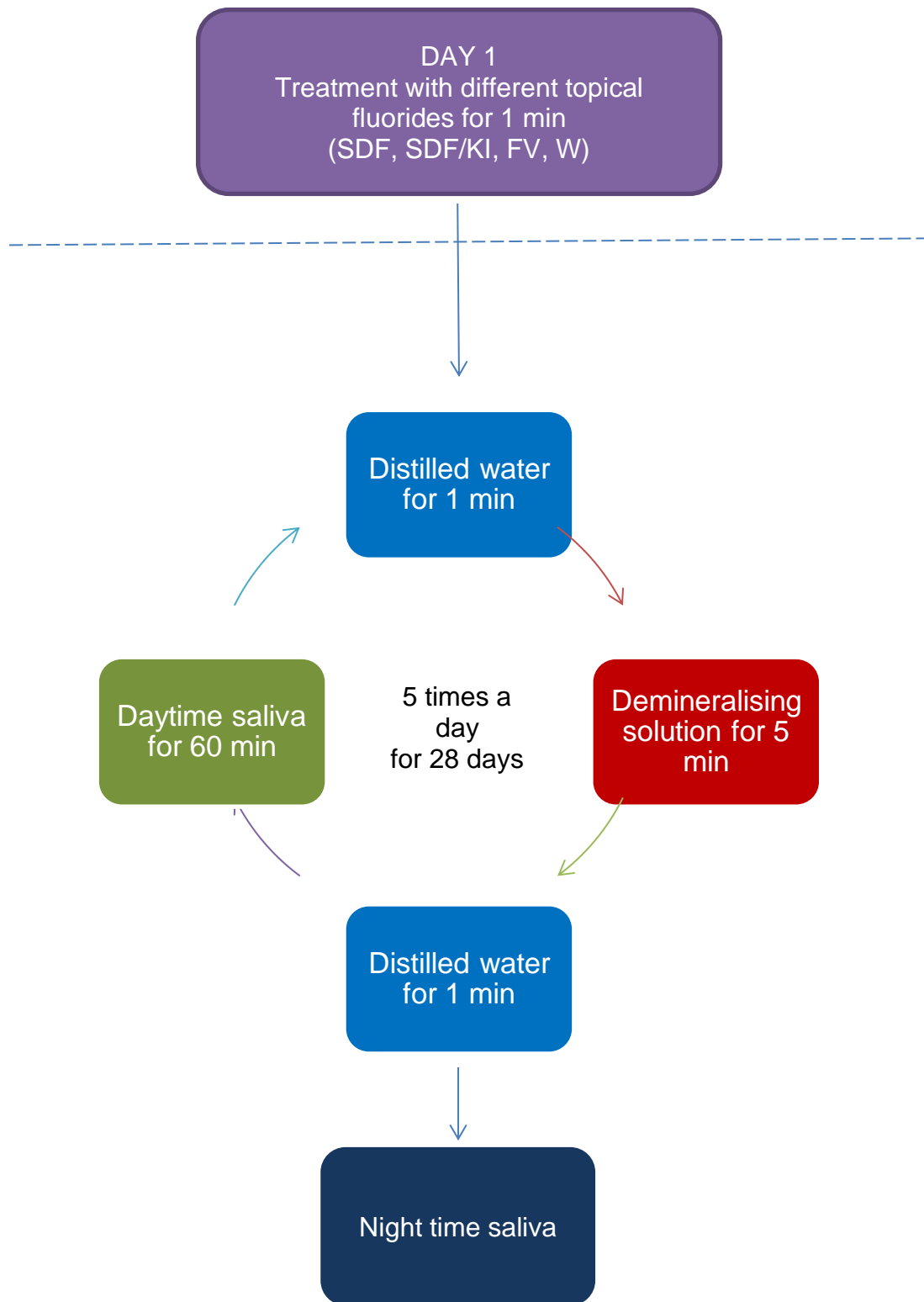


Figure 4-19: Flow chart of pH cycling regime 28 days.

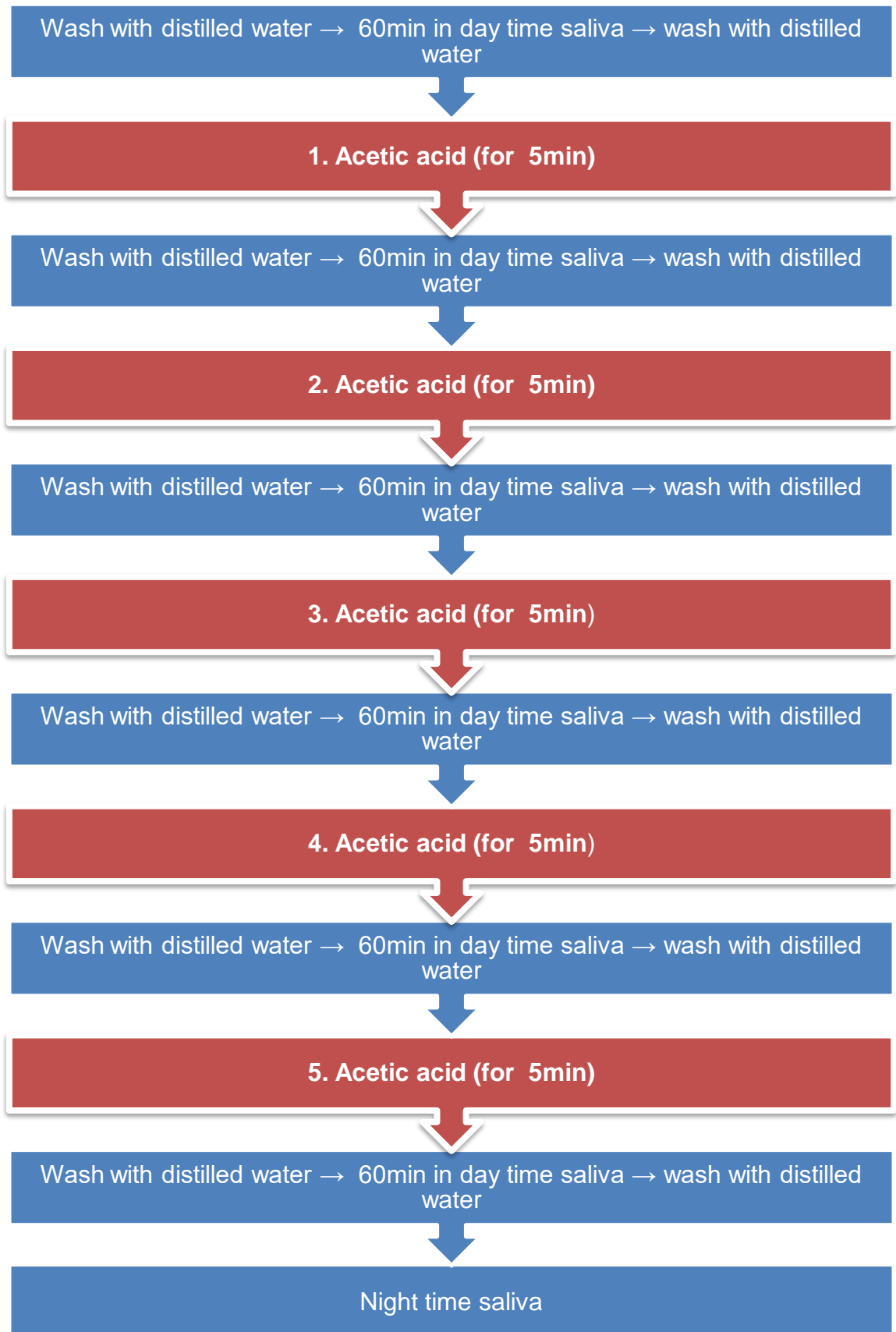


Figure 4-20: Flow-chart of daily pH-cycling regime used in this study

4.11 Preparations of solutions used in the study

4.11.1 Artificial saliva

There are two types of artificial saliva that were used in this study i.e. artificial day and night saliva (Table 4-3 and Table 4-4) . The day saliva was used during the day time during the pH cycling regime, between acid exposures. The night solution was used to store the slabs at night time. The day saliva is supersaturated, which allows remineralisation of dentine slabs. On the other hand, the night saliva is a saturated solution and do not provide any mineral changes.

The saliva solutions were prepared based on the electrolyte composition of natural saliva is recommended to be used to eliminate any precipitation on the lesion surface. This artificial saliva composition was recommended by Dr. Shellis (Department of Oral and Dental Science, University of Bristol, UK).

Table 4-3: Day time saliva formulation.

Salt	Concentration g/L
Calcium carbonate	0.07
Magnesium carbonate (hydrated basic)	0.019
Potassium di-hydrogen phosphate	0.554
HEPES buffer (acid form)	4.77
Potassium chloride	2.24

900 ml distilled water 1.8 ml 1 mol/L HCl and the above components were stirred using a shaker until dissolved. The pH was adjusted to 6.8 by adding KOH solution that is made up to 1L with de-ionised water.

Table 4-4: Night time saliva formulation.

Salt	Concentration g/L
Calcium carbonate	0.05
Magnesium carbonate (hydrated basic)	0.019
Potassium di-hydrogen phosphate	0.068
HEPES buffer (acid form)	4.77
Potassium chloride	2.24

For the night time saliva, 900 ml distilled water 1.8 ml 1 mol/ L HCl and above components were stirred using shaker until they all dissolve. The pH will be adjusted to 6.8 by adding KOH solution that is made up to 1L with de-ionised water.

4.11.2 Demineralising solution

The preparation of demineralising solution according to (ten Cate et al., 2006) is as shown in Table 4-5.

Table 4-5: Demineralising solution constitution 10x stock solution.

Contents	Concentration g/L
Calcium chloride (CaCl₂)	1.665 g
Potassium di-hydrogen phosphate (NaH₂PO₄)	1.13 g
Acetic acid	28.73 ml

The above contents and 1000 ml distilled water were stirred using magnetic stirrer until fully dissolved. This mixture makes 50 mM of concentrated acetic acid. This stock solution was diluted by adding 1 part of stock to 9 parts of deionised water giving a final acetic acid concentration of 5 mM. The pH was adjusted to pH 4.8 by adding KOH and measured by using a pH meter.

4.12 Training and calibration

The study investigator had received training to use the computer-aided Duramin Indenter Machine (Struers A/S, DK 26-10, Denmark) and was familiar with the Duramin software before the study. The investigator's analysis of sound and demineralised dentine by microhardness machine were calibrated.

4.13 Intra-examiner reliability

Approximately 15% of the dentine slabs were randomly selected and retested with CSMH at the end of the experiment. Paired t-test was used in order to allow assessment of intra-examiner reliability.

4.14 Statistical Analysis

SPSS statistical software package (SPSS Inc. ver.22). was used for analysing the data and measure the statistical difference. Descriptive statistics were used to calculate the mean, median, range, and standard deviation. Shapiro-Wilk test was used to test the normality of the data. The data were not normally distributed. Wilcoxon-Signed Rank test used to measure the (p) values to compare within groups.

Kruskal-Wallis test was used to compare between groups. Furthermore, post-hoc pairwise comparison with Bonferroni correction was used to assess if there was any significant difference between groups. The test also calculated the 95% confidence interval. Significance was considered when $p < 0.05$.

Chapter 5 Results

5.1 Dentine Hardness

The Shapiro-Wilk test was carried out to check the normality of the data distribution. The data were not normally distributed as shown in Table 5-1. Therefore, non-parametric Wilcoxon-Signed Rank test was used to assess the change in dentine hardness (KHN) within each group and Kruskal Wallis test was used to determine the difference between groups.

Table 5-1: Test of Normality (dentine hardness-KHN)

Group	SDF		SDF/ KI		FV		W	
	Untreated	After treatment	Untreated	After treatment	Untreated	After treatment	Untreated	After treatment
20	.352	.040*	.000*	.900	.370	.057	.000*	.001*
40	.334	.881	.017*	.083	.024*	.289	.003*	.014*
60	.917	.036*	.116	.280	.257	.147	.002*	.740
80	.304	.464	.140	.505	.658	.544	.056	.049*
100	.937	.507	.550	.697	.704	.497	.892	.100
120	.618	.697	.579	.033*	.034	.257	.206	.922
140	.992	.166	.122	.165	.164	.319	.892	.848
160	.671	.075	.134	.096	.503	.249	.280	.656
180	.479	.706	.266	.180	.108	.931	.825	.396
200	.967	.349	.924	.219	.189	.965	.838	.286
220	.980	.796	.617	.034*	.569	.536	.720	.349
240	.951	.127	.984	.041*	.060	.737	.594	.882
260	.443	.917	.723	.816	.223	.338	.542	.673
280	.820	.548	.609	.989	.196	.572	.832	.249
300	.746	.533	.611	.829	.226	.248	.480	.504

* Shapiro-Wilk test significance $p < 0.05$

5.2 Change in dentine hardness within each group

The dentine Knoop hardness mean values of both untreated and after treatment with their standard deviation for all four groups at each level of indentation were shown in Table 5-2 and Table 5-3. Data were not-independent because the dentine slabs for untreated and after treatment were used from the same tooth, Therefore, Wilcoxon Signed Rank test was performed in order to compare the statistical difference of change in dentine hardness at untreated and after treatment within the same group. The tables also showed some significant change from baseline across all groups according to each depth of indentation from dentine lesion surface.

Table 5-2: Change from baseline (Dentine Knoop Hardness – KHN) for SDF and SDF/KI group.

Level of indentation (µm)		20	40	60	80	100	120	140	160	180	200	220	240	260	280	300
Group Silver Diamine Fluoride (SDF)	Untreated (KHN)	15.42	22.53	27.90	31.99	35.98	37.89	41.01	41.07	43.08	43.94	43.39	45.90	45.82	47.20	47.48
	SD	3.68	5.45	5.49	5.50	5.97	6.49	6.74	7.87	7.03	7.42	6.65	7.13	5.86	6.52	6.33
	Posttreatment (KHN)	27.28	31.44	33.77	34.76	36.58	37.28	38.97	38.52	39.69	40.27	40.24	42.07	42.66	42.86	44.56
	SD	5.91	6.18	5.12	5.70	7.83	6.52	6.07	6.54	5.93	5.17	6.47	7.44	5.79	6.72	6.19
	Mean Difference (KHN)	11.86	8.92	5.87	2.76	0.61	-0.62	-2.04	-2.55	-3.40	-3.67	-3.15	-3.83	-3.15	-4.35	-2.91
	SE difference	1.34	1.29	1.31	1.41	1.88	1.86	1.69	1.71	1.86	1.56	1.57	1.48	1.25	1.58	1.72
	Sign. (2-tailed)	.000*	.000*	.000*	.074	.778	.600	.230	.115	.098	.030*	.093	.030*	.025*	.014*	.206
Group Silver Diamine Fluoride + Potassium Iodide (SDF/KI)	Untreated (KHN)	15.07	21.65	27.05	31.13	34.87	38.64	38.61	41.75	43.50	43.26	44.56	46.22	48.30	47.98	50.65
	SD	5.22	6.42	5.82	5.66	5.78	5.69	4.24	6.43	5.41	4.67	5.14	5.66	4.77	5.93	5.21
	Posttreatment (KHN)	20.80	25.46	29.83	32.31	33.80	35.84	36.54	38.11	38.54	38.09	39.85	40.62	40.65	41.72	43.16
	SD	3.37	4.21	4.81	4.41	4.95	5.30	4.21	4.10	3.82	3.88	4.04	4.54	6.00	4.36	4.79
	Mean Difference (KHN)	5.72	3.80	2.78	1.18	-1.07	-2.80	-2.08	-3.64	-4.97	-5.17	-4.71	-5.60	-7.65	-6.26	-7.49
	SE difference	1.12	1.48	1.58	1.42	1.48	1.50	1.16	1.51	1.33	1.05	1.11	1.53	1.51	1.28	1.32
	Sign. (2-tailed)	.000*	.009*	.104	.382	.427	.088	.061	.026*	.003*	.000*	.001*	.004*	.000*	.000*	.000*

*Wilcoxon Signed Rank Test Significance p<0.05

Table 5-3: Change from baseline (Dentine Knoop Hardness – KHN) for FV and W group.

Level of indentation (µm)		20	40	60	80	100	120	140	160	180	200	220	240	260	280	300
Group Fluoride Varnish (FV)	Untreated (KHN)	13.94	18.87	25.48	30.70	33.32	36.88	42.38	44.34	45.20	46.71	47.33	50.19	50.41	51.30	53.06
	SD	4.32	6.13	7.37	7.08	7.49	6.83	7.38	6.45	7.19	5.26	4.68	4.40	4.90	6.15	3.98
	Posttreatment (KHN)	17.20	22.16	27.42	31.24	33.12	35.51	36.16	38.00	37.56	39.48	40.37	40.10	40.71	42.09	41.86
	SD	3.95	3.99	4.23	4.40	4.87	5.24	4.87	4.73	5.13	4.37	4.33	3.59	4.22	4.51	4.58
	Mean Difference (KHN)	3.25	3.30	1.94	0.54	-0.20	-1.37	-6.22	-6.34	-7.64	-7.24	-6.96	-10.10	-9.70	-9.21	-11.20
	SE difference	1.29	1.63	1.95	1.82	1.97	1.90	1.74	1.62	1.67	1.35	1.15	1.25	1.32	1.67	1.34
	Sign. (2-tailed)	.025*	.058*	.146	.527	.904	.174	.004*	.002*	.001*	.000*	.000*	.000*	.000*	.000*	.000*
Group Distilled Water (W)	Untreated (KHN)	12.96	18.14	24.55	29.15	33.25	37.33	41.26	43.38	45.94	45.59	48.46	48.64	48.01	49.79	51.61
	SD	5.98	8.09	9.89	9.85	8.83	10.36	9.45	9.31	9.07	9.08	10.64	7.87	7.72	7.84	6.77
	Posttreatment (KHN)	11.42	15.54	19.56	24.44	27.32	27.34	30.40	31.28	33.06	33.93	33.26	35.07	34.52	36.34	38.07
	SD	3.71	5.75	5.60	6.83	7.13	5.66	6.54	6.79	5.42	6.43	5.99	7.12	5.56	5.81	6.93
	Mean Difference (KHN)	-1.54	-2.60	-4.99	-4.71	-5.93	-9.99	-10.86	-12.10	-12.88	-11.66	-15.20	-13.58	-13.48	-13.45	-13.54
	SE difference	1.05	1.72	1.90	2.12	2.08	2.27	1.95	2.21	2.00	2.10	2.24	2.23	1.78	1.83	1.51
	Sign. (2-tailed)	.242	.196	.012*	.045*	.023*	.000*	.000*	.000*	.000*	.000*	.000*	.000*	.000*	.000*	.000*

*Wilcoxon Signed Rank Test Significance p<0.05

5.2.1 Change in dentine hardness for SDF group

The hardness of dentine lesions increased after treatment in the SDF group in comparison to the untreated lesions at depths up to 100 μm from the dentine surface, a significant increase at level 20, 40, 60 μm using Wilcoxon Signed Rank test ($p < 0.05$).

However, this trend was reversed and dentine became softer over the course of the pH-cycling regime at depths 120 μm and below. Moreover, at depths 200, 240, 260, 280 μm from lesion surface, dentine was significantly softer ($p < 0.05$) as shown in Figure 5-1 and Figure 5-2.

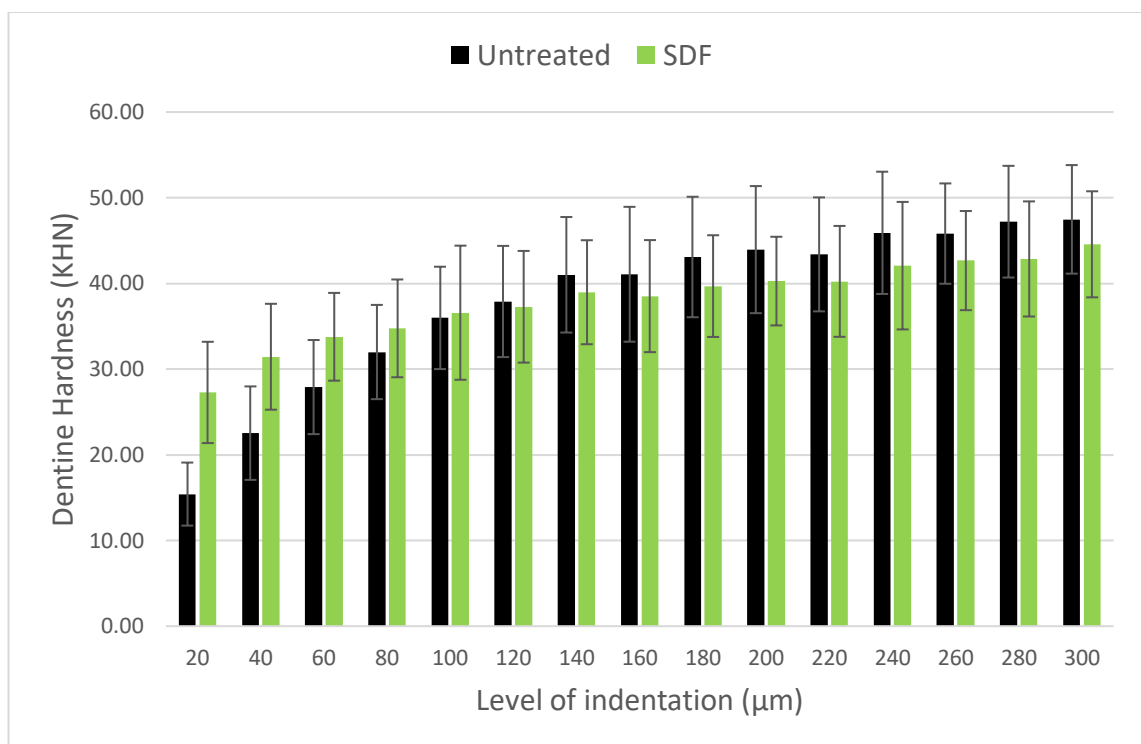


Figure 5-1: Change in dentine hardness after treatment with Silver Diamine Fluoride (SDF).

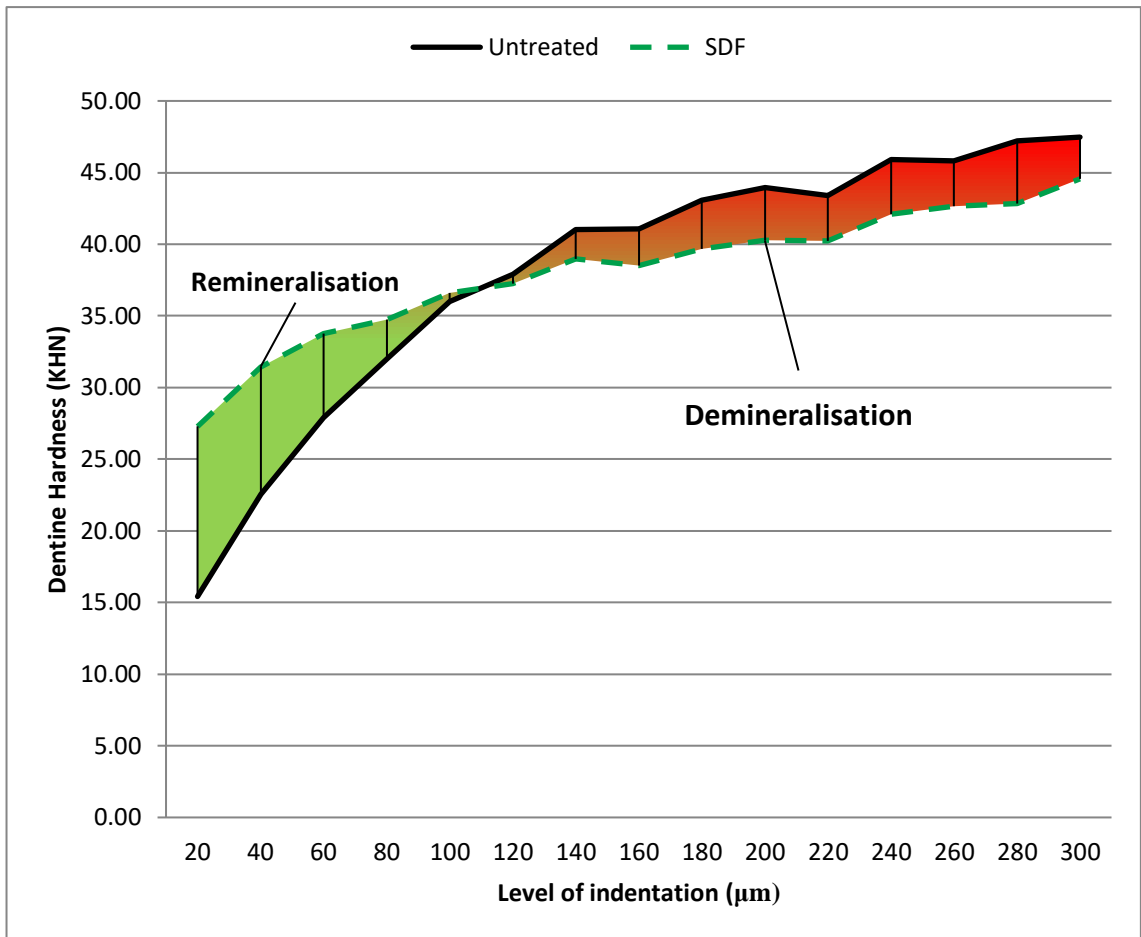


Figure 5-2: The effect of Silver Diamine Fluoride (SDF) on dentine hardness.

5.2.2 Change in dentine hardness for SDF/KI Group

The hardness of dentine lesions increased after treatment in the SDF/KI group in comparison to the untreated lesions at depths up to 80 μm from the dentine surface, a significant increase at level 20, 40 μm using Wilcoxon Signed Rank test ($p < 0.05$).

However, this trend was reversed and dentine became softer over the course of the pH-cycling regime at depths 100 μm and below. Moreover, at depths 160, 180, 200, 220, 240, 260, 280, 300 μm from lesion surface, dentine was significantly softer ($p < 0.05$) as shown in Figure 5-3 and Figure 5-4.

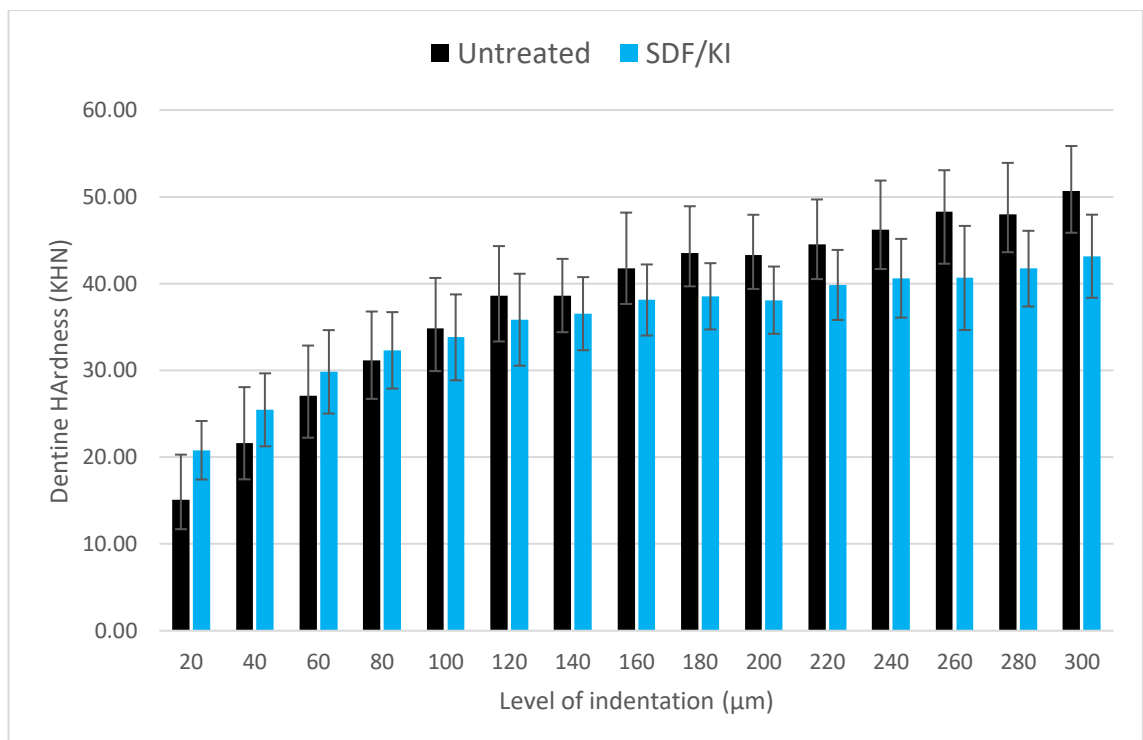


Figure 5-3: Change in dentine hardness (KHN) after treatment with Silver Diamine Fluoride + Potassium iodide (SDF/KI).

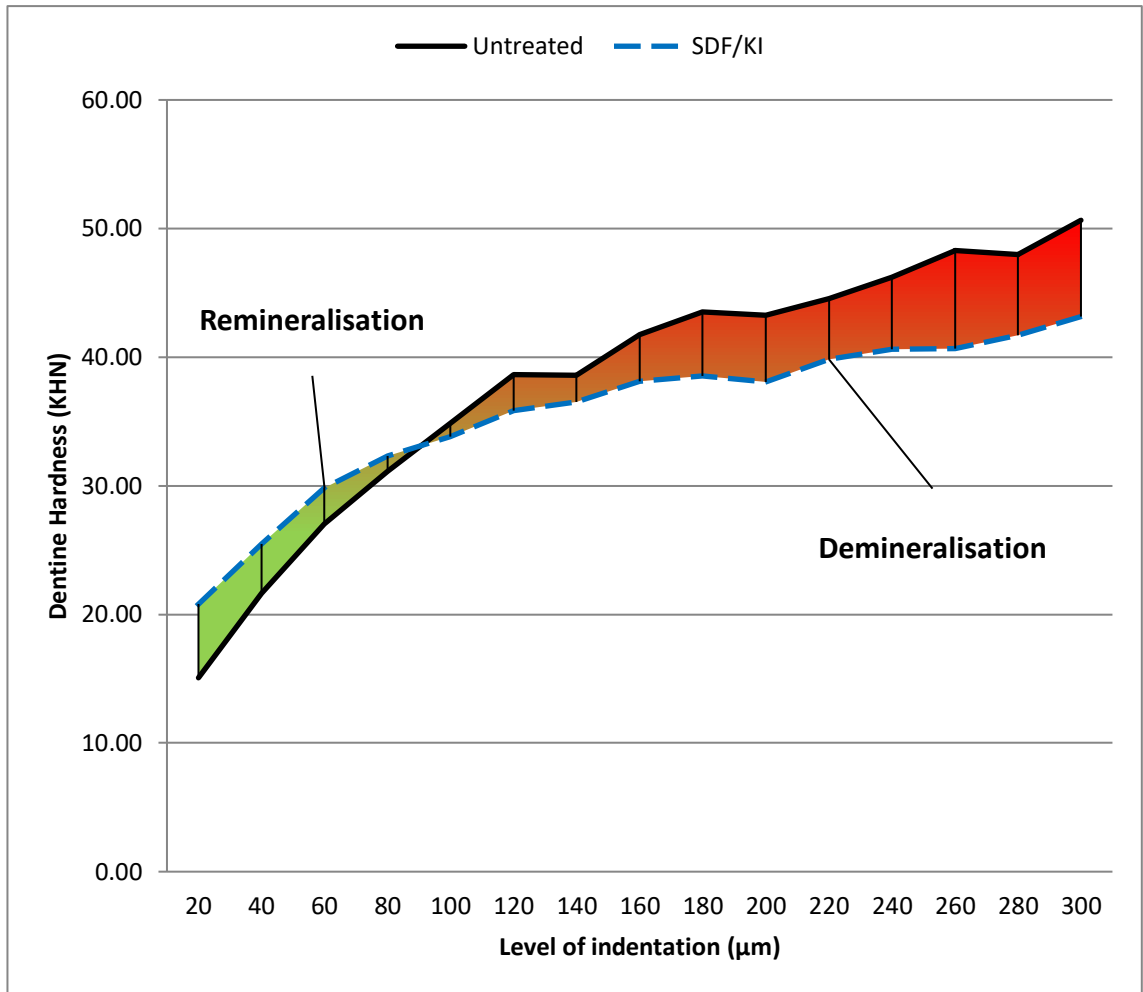


Figure 5-4: Effect of Silver Diamine Fluoride + Potassium Iodide (SDF/KI) on dentine hardness.

5.2.3 Change in dentine hardness for Group FV

The hardness of dentine lesions increased after treatment in the FV group comparison to the untreated lesions at depths up to 80 μm from the dentine surface, a significant increase at level 20, 40 μm using Wilcoxon Signed Rank test ($p < 0.05$).

However, this trend was reversed and dentine became softer over the course of the pH-cycling regime at depths 100 μm and below. Moreover, at depths 140, 160, 180, 200, 220, 240, 260, 280, 300 μm from lesion surface, dentine was significantly softer ($p < 0.05$) as shown in Figure 5-5 and Figure 5-6.

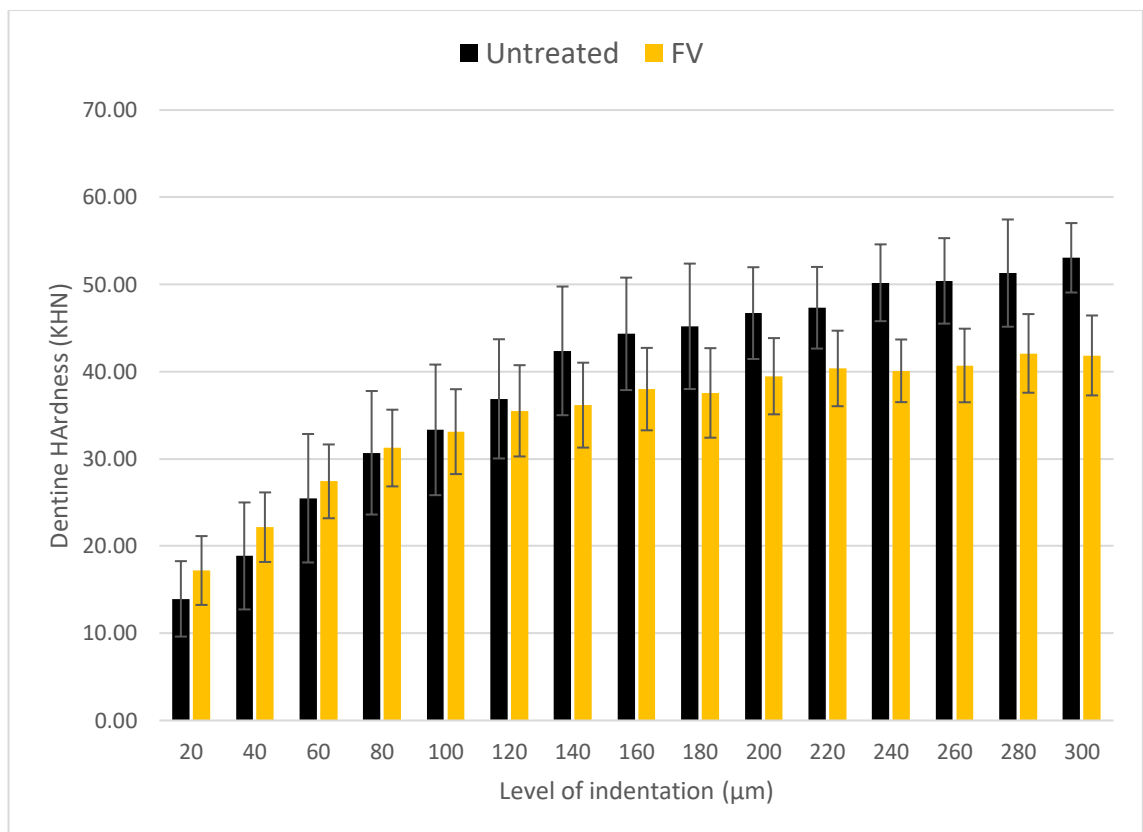


Figure 5-5: Change in dentine hardness (KHN) after treatment with Fluoride Varnish (FV).

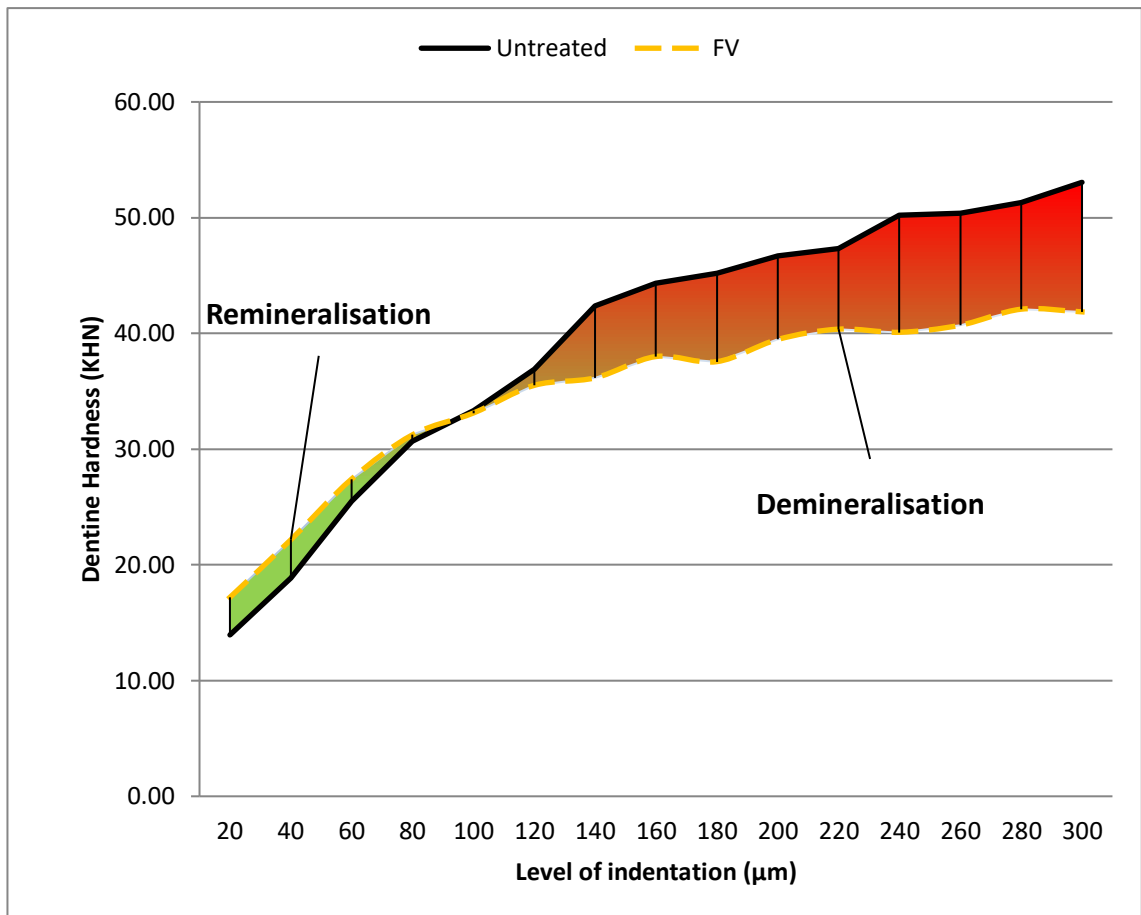


Figure 5-6: Effect of Fluoride varnish (FV) on dentine hardness.

5.2.4 Change in dentine hardness for Group W

In the W group, the hardness of dentine lesions decreased significantly after treatment at all depths. However, the decrease in hardness at level 20 and 40 μm was not significant using Wilcoxon Signed Rank test ($p < 0.05$). This change was shown in Figure 5-7 and Figure 5-8.

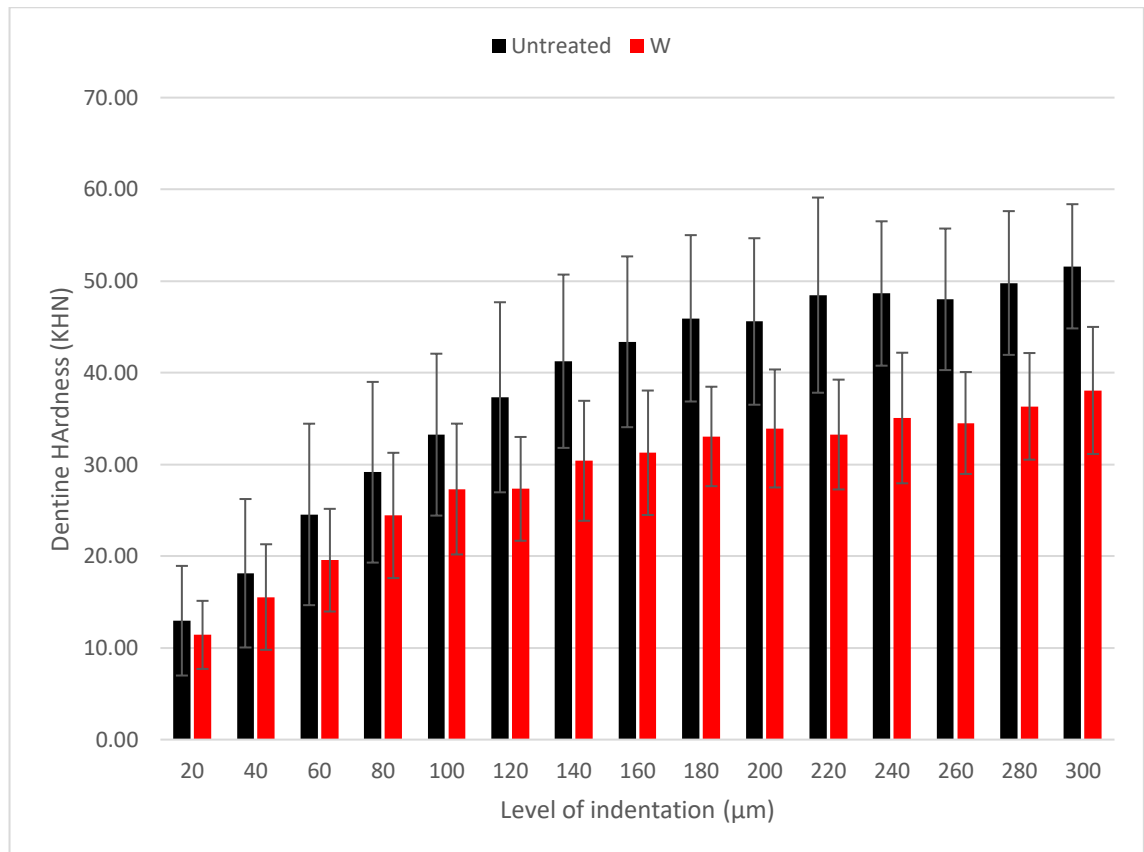


Figure 5-7: Change in dentine hardness (KHN) after treatment with deionised water (W).

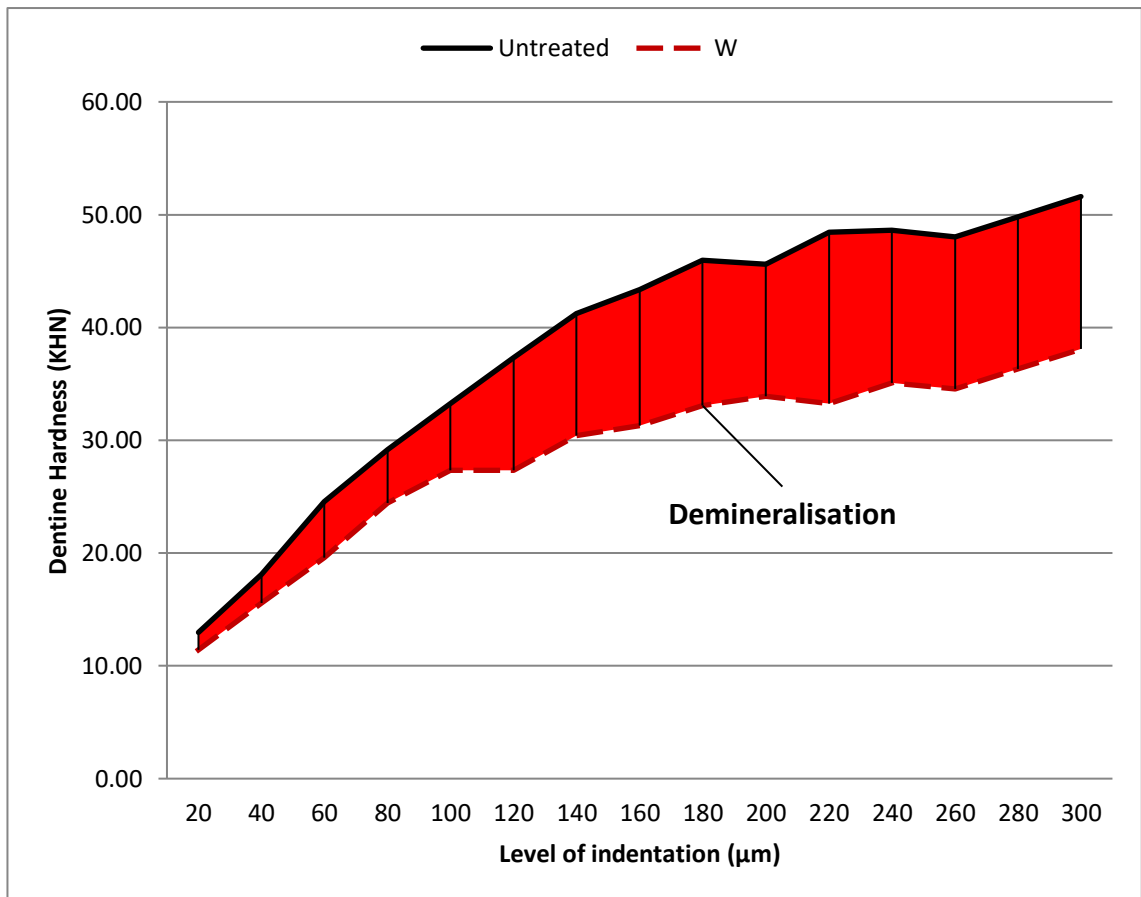


Figure 5-8: Effect of deionised water (W) on dentine hardness.

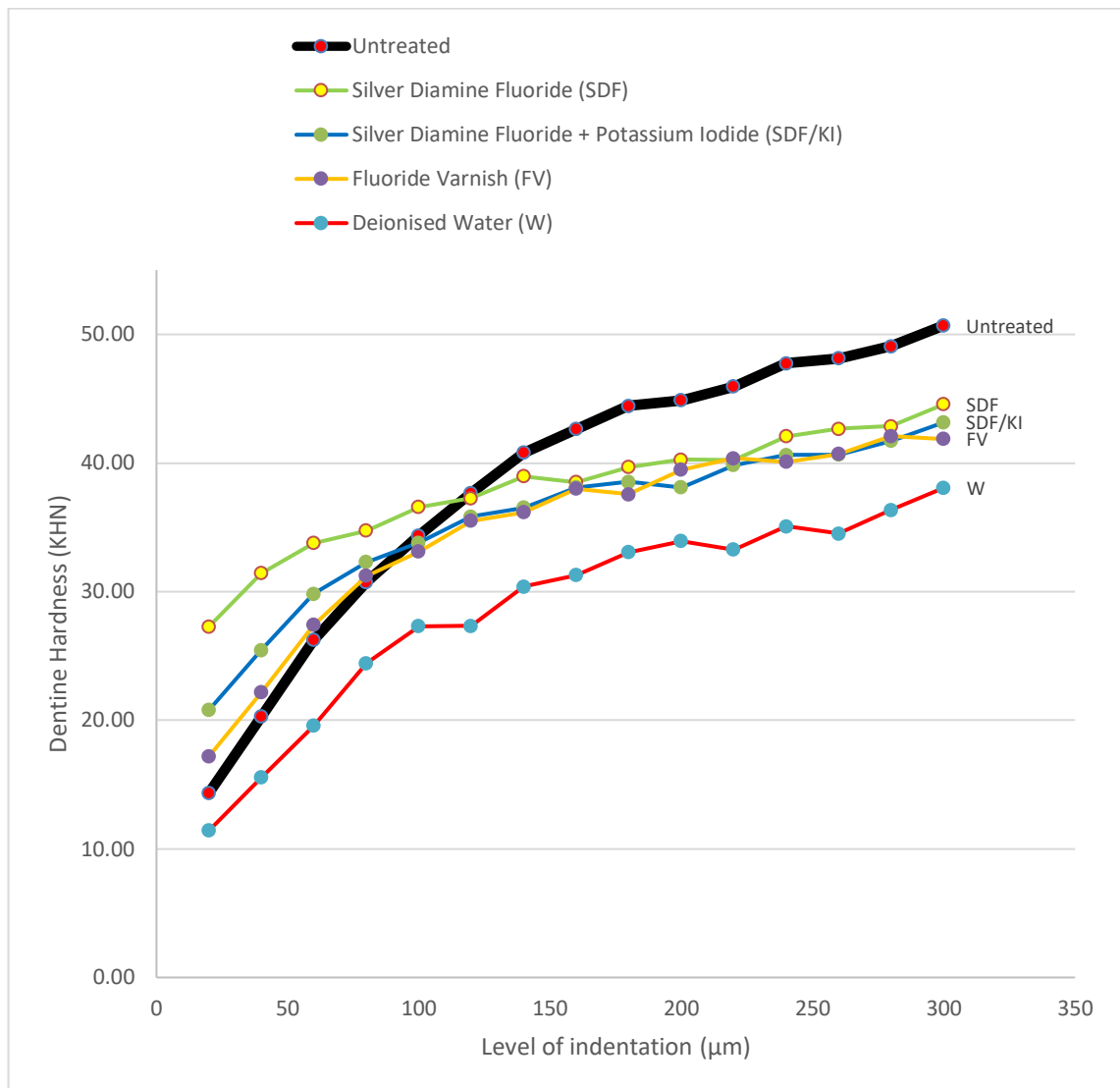


Figure 5-9: Overall change of dentine hardness (KHN) of the lesions in all groups.

As a summary, the dentine hardness in untreated group showed an increase in trend when going deeper into the lesion (Figure 5-9), as well as all other groups. There was an increase in dentine Knoop hardness (KHN) after treatment with SDF, SDF/KI and FV groups from level 20 to 80 µm. There was a statistically significant increase in dentine hardness of dentine lesions from baseline after treatment with: SDF at level 20, 40, 60 µm; SDF/KI at level 20, 40 µm; and FV level 20, 40 µm. The mean of dentine Knoop hardness were all decreased from baseline from level 120 to 300 µm for all groups. There was no improvement in mean of dentine hardness in the water group and the reduction were statistically significant from level 60 µm onwards. No significant change in dentine Knoop hardness from baseline was seen at level 80, 100, and 120 µm for all groups except Group W.

5.3 Difference between groups

The Dentine hardness difference was measured using the following formula:

Mean Difference in dentine hardness	=	Mean dentine hardness after treatment	-	Mean dentine hardness at baseline
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Table 5-2, Table 5-3 and Figure 5-10 show the difference in mean dentine hardness between the four groups. A positive value indicated an increase in dentine hardness and a negative value indicated a reduction in dentine hardness. There was an increase in dentine hardness for all groups from level 20 until level 80 μm for all groups except for Group W. The highest increase in dentine hardness was seen in Group SDF at level 20 (11.86 ± 1.34 KHN) followed by Group SDF/KI (5.72 ± 1.12 KHN) and FV (3.25 ± 1.29 KHN). Group W showed a small reduction in dentine hardness at level 20 μm (-1.54 ± 1.05 KHN) increase in mean difference of dentine hardness from level 20 until level 300 μm . However, the difference was increasingly negative. The largest reduction in dentine hardness was found at level 220 when treated with W (-15.20 ± 2.23 KHN).

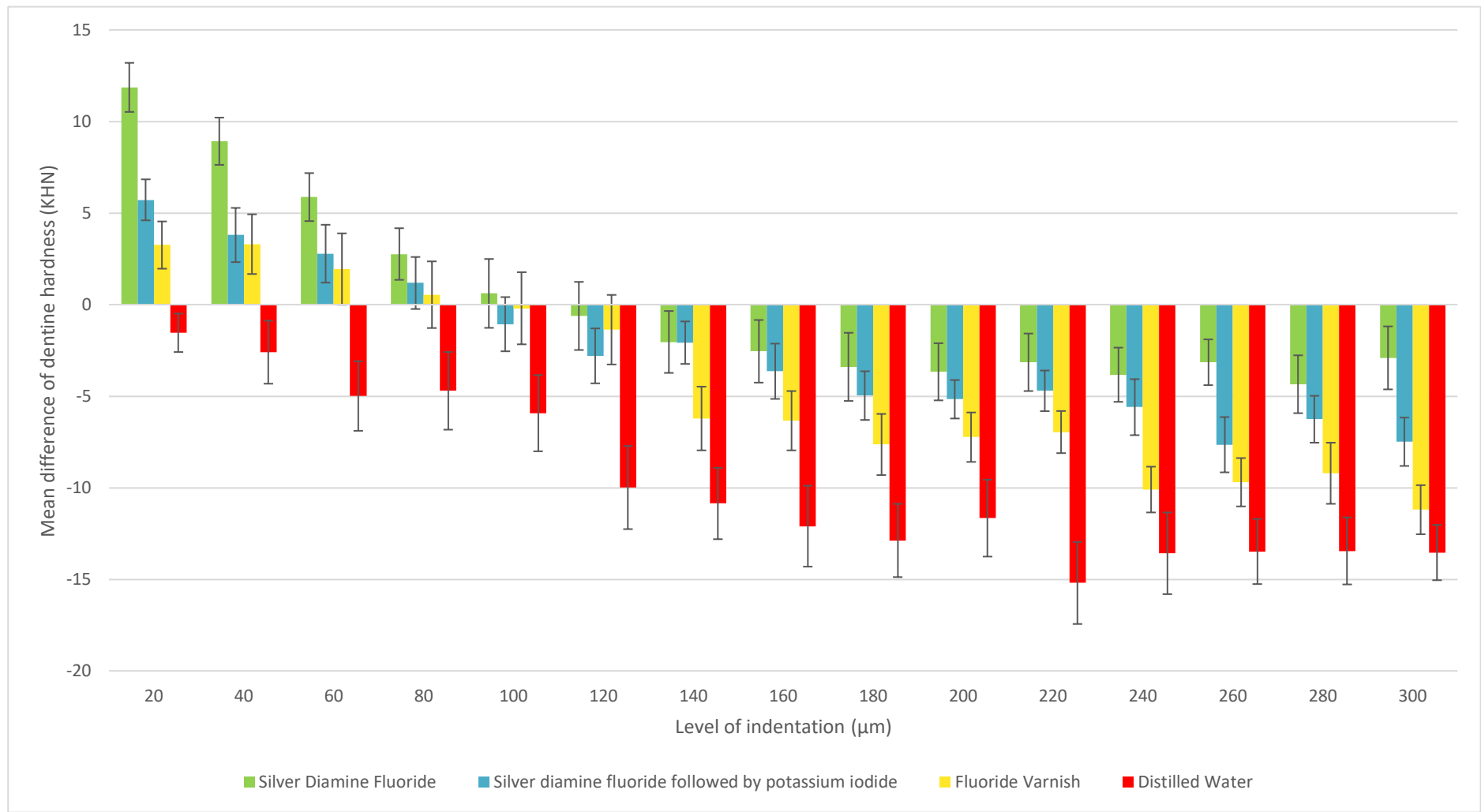


Figure 5-10: Mean difference in dentine hardness between groups.

The box-and-whisker plots show the median, maximum and minimum values the first and third quartiles within the data set. The line in the box of the box-and-whisker plot was the median value for the data. Also, the presence of outliers within each group at each level of indentation is demonstrated (Figure 5-11).

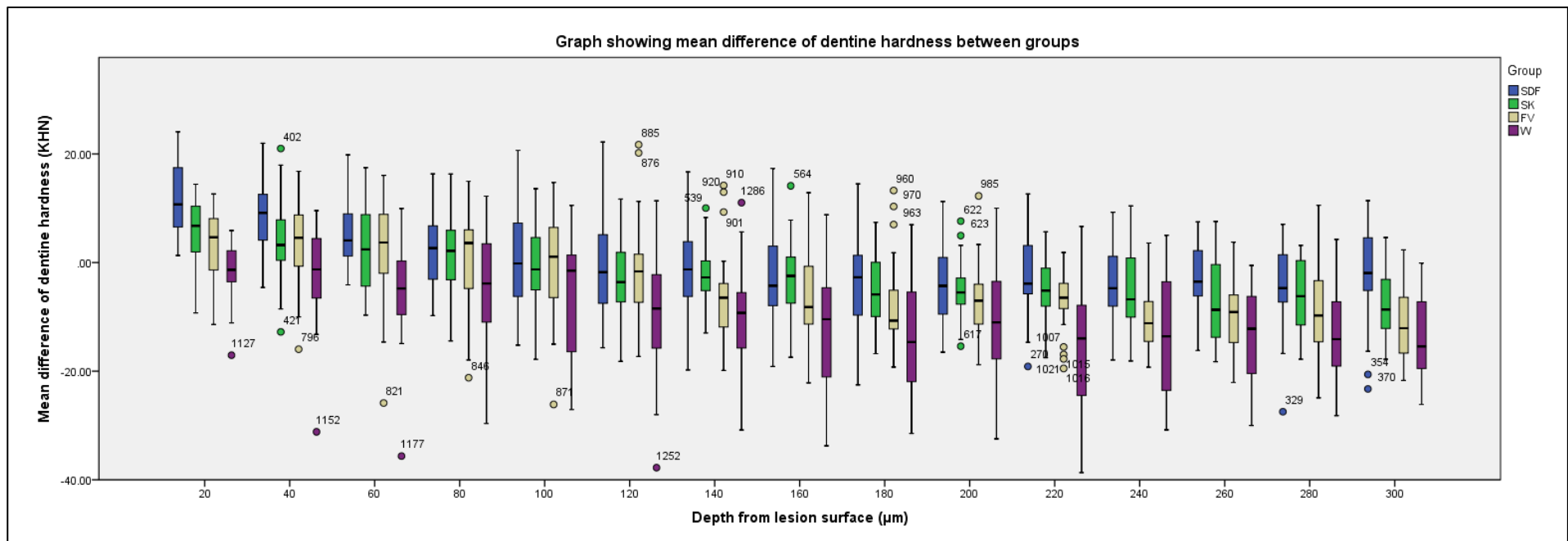


Figure 5-11: Box-and-whisker plots of mean difference of dentine hardness across groups.

5.3.1 Difference between levels of indentations between groups

Non parametric Kruskal-Wallis Test was conducted to determine if the difference in dentine hardness at every level of indentation was statistically different between the four groups. It shows that the mean difference of dentine hardness was statistically significant between each level of indentation except Level 80 and 100 as shown in Table 5-4. Pairwise comparison with Bonferroni correction was performed as shown in Table 5-5. Pairwise comparison was not calculated when there was no significance difference as seen on Level 80 and 100. The post hoc analysis revealed statistically significant differences of mean difference between Group SDF and FV at level 20 and 300 μm ($p < 0.01$); Group SDF and W at all levels except 80 and 100 ($p < 0.01$); Group SDF/KI and W at level 20, 60, 160, 180 and 220 μm ; and between Group FV and W at level 60 μm only. The difference of dentine hardness between group SDF and SDF/KI was not significant. There was no statistical difference found between all groups at level 80 and 100 μm , therefore they were not analysed for pairwise comparison.

Table 5-4: Kruskal-Wallis test for mean difference between four groups.

Level of indentation (μm)	Kruskal-Wallis Test Sig. between groups
20	0.000*
40	0.000*
60	0.000*
80	0.069
100	0.174
120	0.015*
140	0.000*
160	0.008*
180	0.004*
200	0.013*
220	0.000*
240	0.002*
260	0.000*
280	0.001*
300	0.000*

*Significance level $p < 0.05$

Table 5-5: Pairwise comparison with Bonferroni test for all groups.

Level of indentation (μm)	Groups					
	W vs SDF	W vs SDF/KI	W vs FV	SDF vs SDF/KI	SDF vs FV	FV vs SDF/KI
20	0.000*	0.001*	0.065	0.105	0.001*	1.000
40	0.000*	0.057	0.135	0.069	0.159	1.000
60	0.000*	0.022*	0.025*	1.000	1.000	1.000
80	n.c	n.c	n.c	n.c	n.c	n.c
100	n.c	n.c	n.c	n.c	n.c	n.c
120	0.021*	0.065	0.124	1.000	1.000	1.000
140	0.003*	0.001	1.000	1.000	0.205	0.118
160	0.012*	0.034*	0.727	1.000	0.734	1.000
180	0.008*	0.031*	1.000	1.000	0.271	0.689
200	0.015*	0.071	0.932	1.000	0.656	1.000
220	0.000*	0.000*	0.067	1.000	0.615	1.000
240	0.005*	0.058	1.000	1.000	0.033*	0.275
260	0.000*	0.215	1.000	0.187	0.014*	1.000
280	0.001*	0.031*	0.842	1.000	0.131	1.000
300	0.000*	0.095	1.000	0.304	0.003*	0.755

*Significance level $p < 0.05$
n.c. = not calculated

5.4 Intra-examiner reliability

Fifteen (n=15) of the treated samples were randomly selected and cross-sectional microhardness was repeated on each slab for 3 times. The mean and standard deviation of Knoop hardness of the dentine lesions were calculated. Paired t-test was conducted to assess the intra-examiner reliability. Table 5-6 shows no significant different between each slab at every level.

Table 5-6: Paired Samples Test for intraexaminer reliability

Level of indentation (μm)	Paired Differences					Sig. (2-tailed)
	Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference		
				Lower	Upper	
20	0.11	2.38	0.66	-1.33	1.55	0.87
40	-0.06	1.48	0.41	-0.95	0.84	0.89
60	-0.64	2.37	0.66	-2.08	0.79	0.35
80	-0.07	1.51	0.42	-0.98	0.84	0.88
100	0.05	1.76	0.49	-1.01	1.12	0.92
120	-0.86	2.97	0.82	-2.66	0.93	0.32
140	-0.78	1.73	0.48	-1.82	0.27	0.13
160	-0.21	1.71	0.48	-1.25	0.82	0.66
180	-1.41	4.68	1.30	-4.24	1.42	0.30
200	-0.85	2.90	0.80	-2.61	0.90	0.31
220	-1.43	4.45	1.24	-4.12	1.26	0.27
240	-0.79	1.54	0.43	-1.72	0.15	0.09
260	-0.86	4.21	1.17	-3.40	1.68	0.47
280	-1.27	3.06	0.85	-3.12	0.58	0.16
300	-1.17	4.03	1.12	-3.60	1.27	0.32

5.6 Surface Morphology - Scanning Electron Microscopy (SEM)

Scanning electron microscopy images of the dentine artificial subsurface lesion were taken for Group W, SDF, SDF/KI.

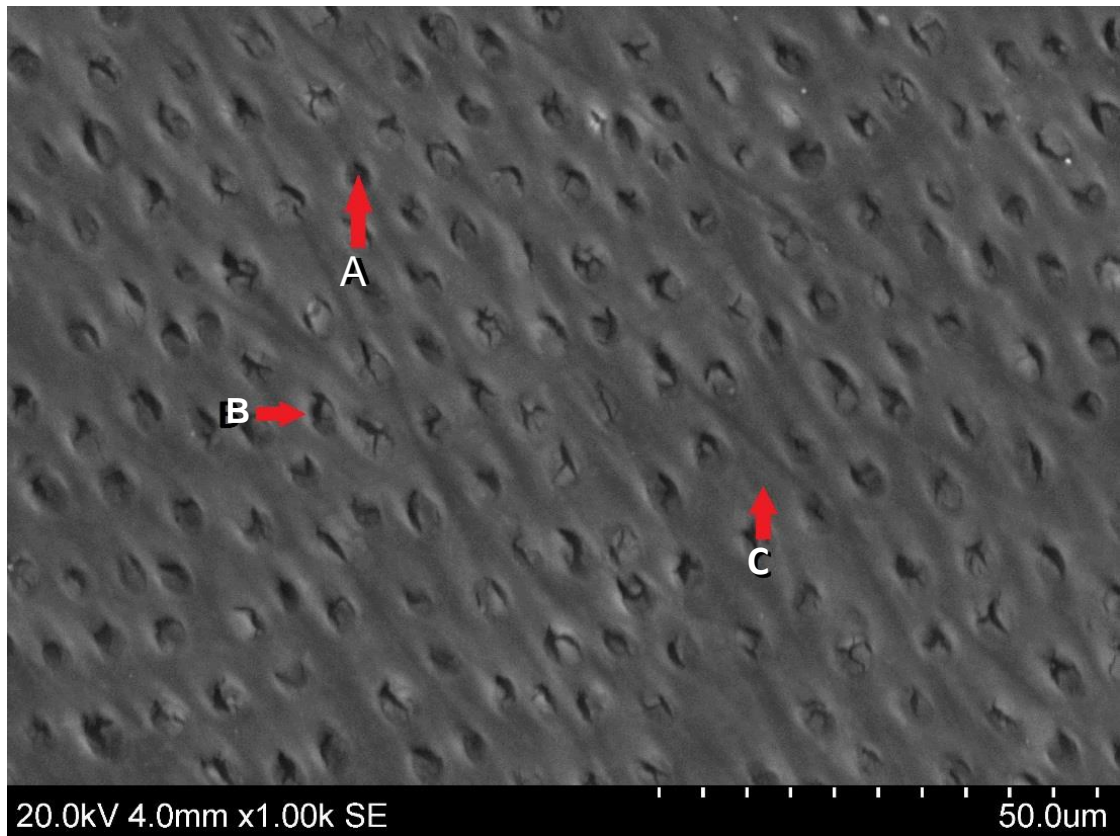


Figure 5-12: SEM image: surface morphology of untreated dentine artificial subsurface lesion. 1000x magnification view.

A – peritubular dentine.

B – dentinal tubule.

C – intertubular dentine.

Figure 5-12 shows the untreated dentine artificial subsurface lesion with the partially exposed dentine tubules. The intertubular dentine appeared smooth and the dentinal tubules were not well demarcated.

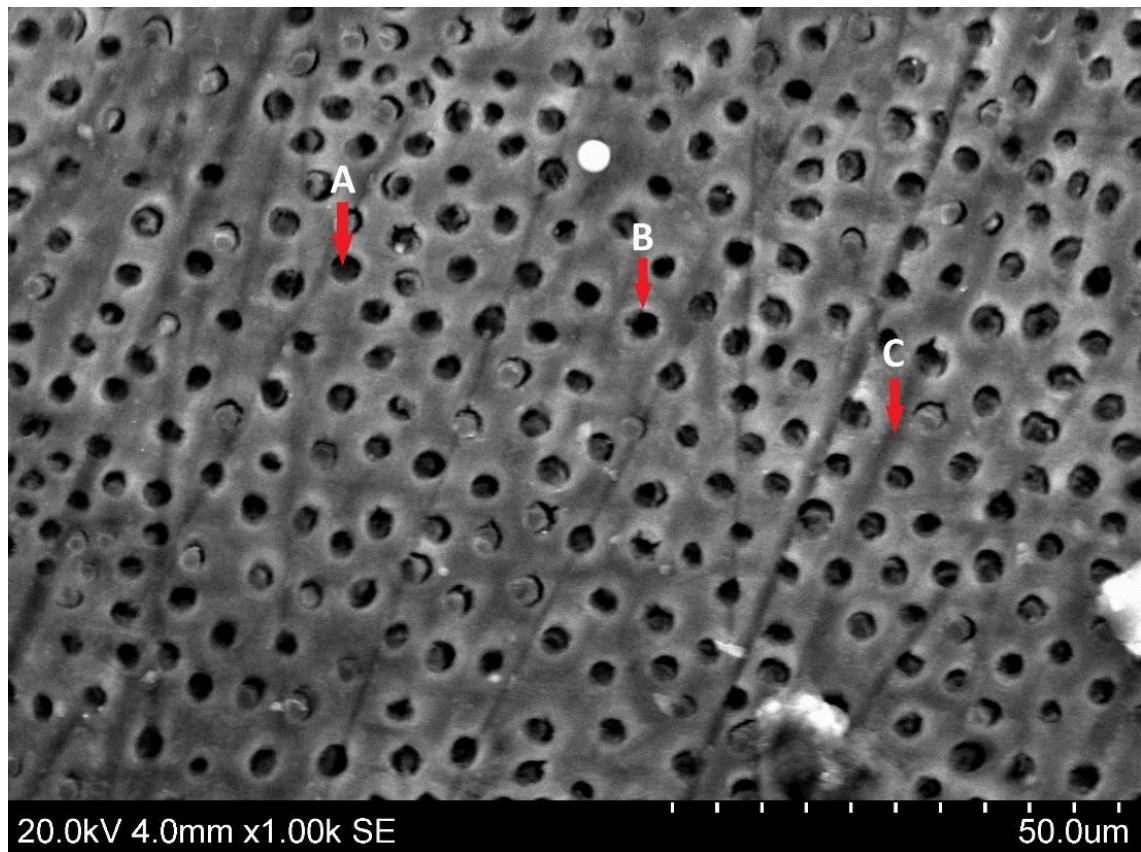


Figure 5-13: SEM image: surface morphology of dentine artificial subsurface lesion. 1000x magnification view of dentine artificial subsurface lesion treated with deionised water (W).
A – peritubular dentine
B – dentinal tubule
C – intertubular dentine

Well demarcated and mostly exposed dentine tubules were seen in Group W. Some of the dentinal tubules were partially blocked (Figure 5-13).

Figure 5-14 and Figure 5-15 show lesions a dense layer of granular precipitation covering part of the lesion. In the exposed part of the lesion, the dentinal tubules were not well defined and occluded with granular particles. There was also presence of irregular shaped debris over the surface.

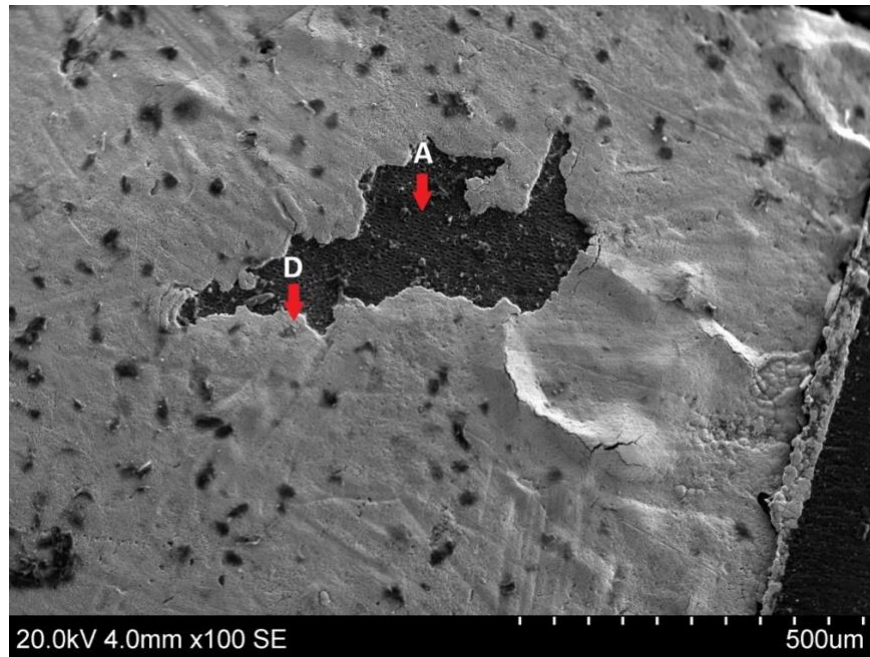


Figure 5-14: SEM image: surface morphology of dentine artificial subsurface lesion treated with silver diamine fluoride. 100x magnification view.

A – dentinal tubules
B – peritubular dentine

C – intertubular dentine
D – silver diamine fluoride layer

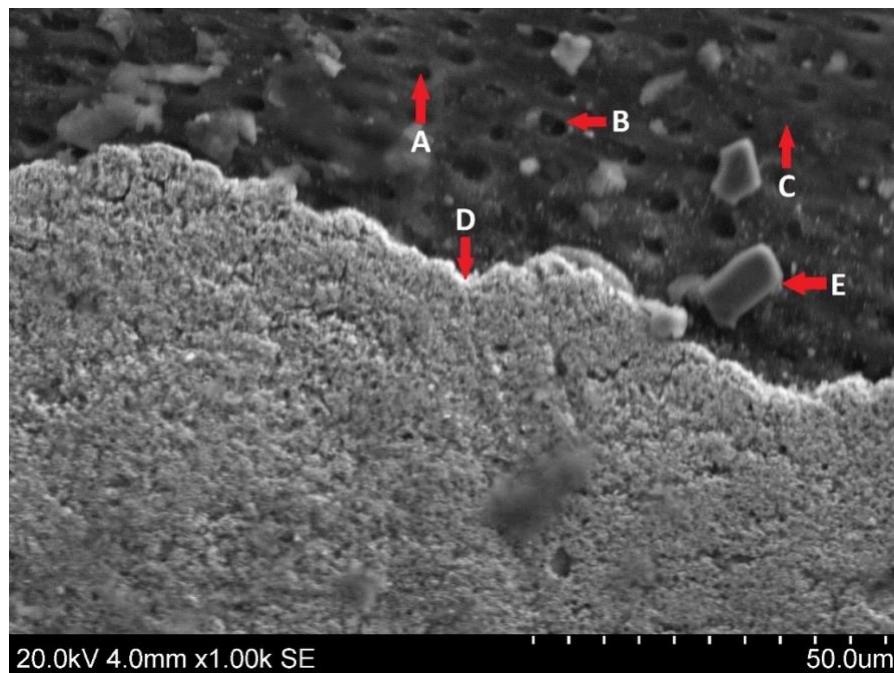


Figure 5-15: SEM image: surface morphology of dentine artificial subsurface lesion treated with silver diamine fluoride. 1000x magnification view.

A – dentinal tubules
B – peritubular dentine
C – intertubular dentine

D – silver diamine fluoride layer
E – debris

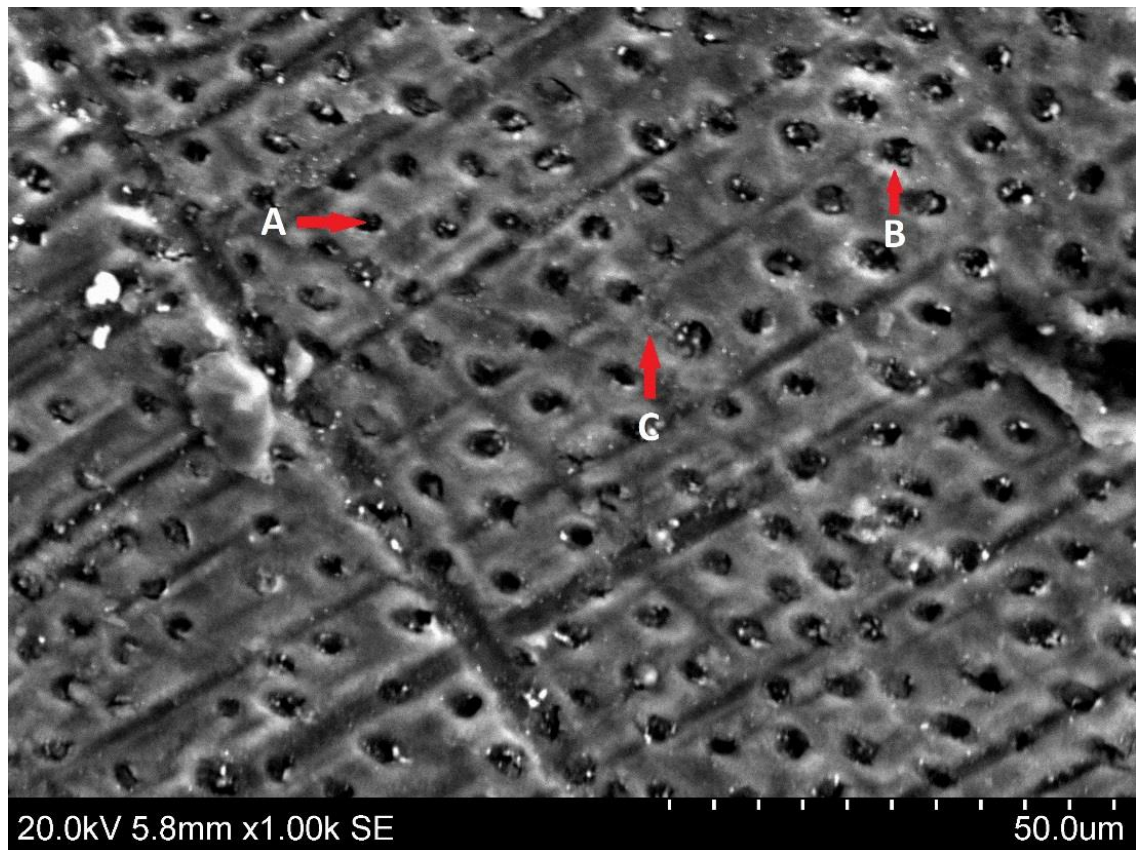


Figure 5-16: SEM image: surface morphology of dentine artificial subsurface lesion treated with SDF followed by potassium iodide. 1000x magnification view.

- A – peritubular dentine
- B – dentinal tubule
- C – intertubular dentine

No layer of precipitation seen over the dentine artificial subsurface lesion in Group SDF/KI but the dentine tubules appeared partially blocked with some dense granules as seen in Figure 5-16.

The size of exposed dentine tubules appeared to be larger in Group W when compared to untreated, Group SDF and SDF/KI.

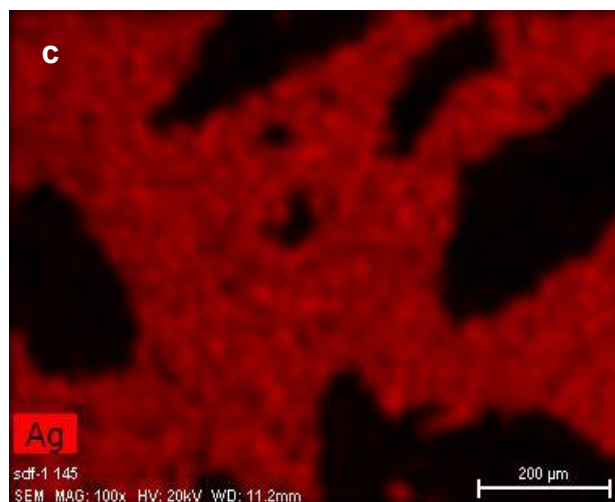
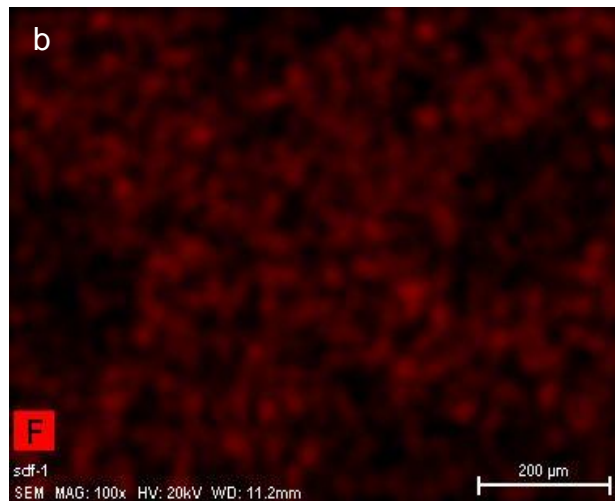
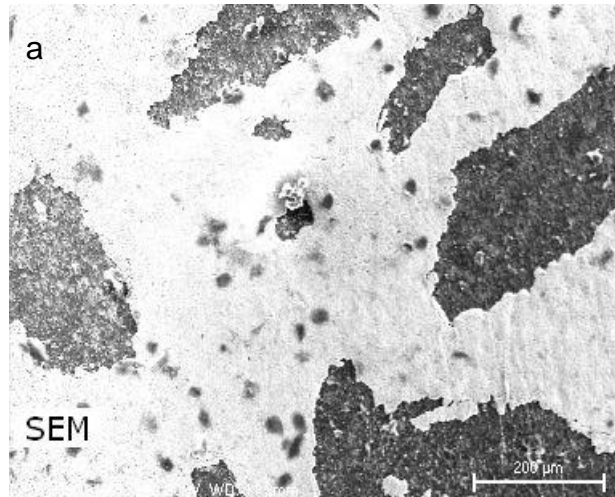


Figure 5-17: (a) SEM image of surface morphology of artificial lesion treated with SDF 100x magnification; (b) Fluoride elemental mapping of dentine artificial subsurface lesion treated with SDF; (c) Silver elemental mapping of dentine artificial subsurface lesion treated with SDF.

F: Fluoride

Ag: Argentum (Silver)

Figure 5-17 (a) shows an image of the SDF layer over the dentine artificial subsurface lesion with some patches (dark zone) present under SEM with 100x magnification. The red zone in Figure 5-17(b) shows presence of fluoride and Figure 5-17(c) shows presence of silver over the dentine artificial subsurface lesion treated with SDF. The areas with less Ag and F density correspond to the dark zones (Figure 5-17(a)).

Chapter 6 Discussion

The important role of topical fluorides in successfully arresting dental caries has been widely studied. A considerable amount of systematic and Cochrane reviews has been published on the potential of remineralisation of enamel and dentine lesion by various topically applied fluoride products (Gao et al., 2016, Marinho et al., 2013, Twetman et al., 2003, Marinho et al., 2003d, Marinho et al., 2003b, Marinho et al., 2003c, Marinho et al., 2003a). However, the search of the literature revealed only a small number of evidence was found associating silver diamine fluoride with its remineralising potential in dental caries in permanent and primary teeth (Chu et al., 2002, Yee et al., 2009, Llodra et al., 2005). In the literature, only a number of *in vitro* studies that compared the effect of SDF and FV on progression and/or regression of dental caries (Zhao et al., 2018). The mechanism of action of SDF is unclear due to the significant variation of the study design, objectives, experimental conditions, model systems and conclusions (Zhao et al., 2018).

6.1 *In vitro* model

This study utilises an *in vitro* model to investigate dentine demineralisation using different topical fluoride formulations. Reflecting to the review of literature, the anti-caries effect of SDF has shown its effectiveness through many clinical studies (Chu et al., 2002, Chu and Lo, 2008a, Craig et al., 2013) *In vitro* model mimics many features of the natural occurrences of carious lesion in the human teeth, such as the pH fluctuation and temperature. However, it is difficult to replicate the actual caries formation that involves the action inorganic proteins from the saliva, normal oral flora and cleansing action through this model (White, 1995, White, 1992, ten Cate, 1990). It is important to regard that any lesion produced in the laboratory is not exactly the same to the caries lesion occurred in the human oral environment and should be referred to “caries-like” lesions (Robinson, 1995). The *in vitro* model can be modified to suit different studies in terms of the hypotheses, aim, methods and conclusions.

Despite the disadvantages of *in vitro* model, this model has the capability to produce single variable experiments under exceedingly controlled conditions and. *In vitro* model also permits the utilisation of a wide scope of analytical methods for substrate analysis, cost less financially and less ethical issues compared to that of *in vivo* model white (White, 1995).

6.2 Study design

This was a one-phase *in vitro* study design to investigate the mineral content of the artificial dentine lesions under pH cycling conditions using different clinically used topical fluoride agents: silver diamine fluoride (SDF), silver diamine fluoride (SDF/KI), sodium fluoride varnish (FV), and distilled water (W) as the negative control.

This study was randomised and non-blinded design due to different methods of application among all groups and staining effect from SDF application in SDF and SDF/KI group.

6.3 Dentine slabs preparation and storage

In the present study, the dentine slabs were prepared from the labial section of the bovine incisor teeth to allow a more uniform thickness and flat surfaces from a method that was described by Arends et al. (1989) and ten Cate et al. (1995). The bovine teeth were stored in 0.1% thymol (Sigma Aldrich) before the dentine slabs preparation with the aim of inhibition of the bacterial growth and prevention of teeth dehydration. Thymol is a plant-derived antimicrobial agent that has the ability to destroy the oral bacteria cell membranes and therefore eliminates certain pathogens that may be present on the teeth (Shapiro and Guggenheim, 1995). Although 0.1% thymol is effective in removing *Streptococcus mutans* from dentine samples, it produces unreliable effects on further demineralisation and remineralisation process (Preston et al., 2007). Therefore, the dentine slabs were transferred and stored in distilled water after the dentine slabs were prepared to prevent dehydration.

The dentine slabs were embedded in custom-made acrylic resins blocks to ease the process of cutting and cross-sectional microhardness analysis. This method of sample preparation was originally designed for this present study.

6.4 Artificial caries lesions

Acidified hydroxyethyl cellulose gel was used to create lesions in the bovine dentine slabs. This gel has the ability to creating lesions with a consistent depth of demineralisations in enamel (Issa, 2004) and more controlled demineralisation process and allow re-precipitation of dissolved mineral ions to create an intact surface layer of the lesion that mimics the caries process (Amaechi et al., 1998). In this present pilot study, the gel was also found to be able to a create consistent lesion in dentine.

In the current study, acidified hydroxyethyl cellulose gel was used to create a sub-surface caries-like lesion. A pilot study was done to determine the depth of lesion by immersing the bovine dentine slabs in the acidified gel for 120 hours in order to produce dentine lesion with approximately 160 μm depth. The hardness of the dentine lesion was determined by cross-sectional microhardness at every 20 μm from the lesion surface.

6.5 pH cycling regime

In the current study, after artificial lesions were created in the dentine slabs, they were exposed to 5 acidic challenges per day for 28 days In order to allow sufficient time to produce demineralisation in the dentine slabs. pH cycling method was initially designed to produce artificial lesions in enamel (Featherstone et al., 1983, Featherstone and Zero, 1992, Stookey et al., 2011). A study compared the use of various methods to creating artificial lesions in dentine, i.e. induction by acidified gel, pH cycling and microbiological methods (Marquezan et al., 2009). The authors found that caries induction by pH cycling produced lesions with similar hardness values to naturally occurring dentine caries up to 40 μm depth. The study subjected the dentine lesion to 14 days of pH cycling as opposed to 28 days which was used in this present study where we were able to assess significant changes in dentine hardness at 300 μm depth.

The topical fluoride agents were applied at the beginning of the pH cycling once, followed by rinsing in distilled water for 1 minute and placed in day time artificial saliva. The dentine slabs went for the first demineralisation challenge by immersion in acetic acid (pH 4.8) for 5 minutes, rinsed with distilled water for 1 minute and placed in day time saliva for 1 hour. This process was repeated for five times, simulating the acid attack in the cariogenic challenges in the oral cavity (Al-Mullahi and Toumba, 2010, Duggal et al., 2001). At the end of the cycle, the dentine slabs were placed in night-time artificial saliva and stored in an incubator at 37°C.

The supersaturated day time saliva was used to allow remineralisation of the dentine slabs with artificial dentine lesions during the day between acid challenges. Conversely, the night-time saliva was a saturated solution that maintains the dentine condition in equilibrium when the dentine slabs were stored overnight (Abdullah, 2009).

The pH cycling protocol used for the current study was adopted and developed at the University of Leeds and has been used in previous caries studies at the Paediatric Dentistry Department (Malinowski, 2010, Al-Kandari, 2017).

6.6 Cross-sectional microhardness

In the present study, the technique for Knoop Cross-sectional microhardness measurement was standardised and was done in a controlled environment for all dentine slabs. The manufacturer trained the investigator to be familiarised with the Duramin software and technique for indentation prior to study commencement in order to minimise the risk of bias.

The pilot study conducted prior to this study has given some light regarding impracticability of using QLF technology in assessing caries after treatment with silver diamine fluoride in enamel due to the inconsistent parameters produced and unable to detect the fluorescence in dentine lesions. Transverse Micro Radiography (TMR) could be used for the method of analysis because it gives more detailed information about the behaviour of the lesion. For example, TMR

could assess the mineral content of the lesion at every 2 μm (Magalhaes et al., 2009). However, both of this method is time-consuming and very expensive. Ideally, the micro-CT scan would have been a better option for analyzing the lesion behaviour in greater detail. Micro-CT is such a sensitive tool in assessing mineral changes in hard tissue research (Swain and Xue, 2009). However, very little data was found regarding the feasibility of micro-CT for analyzing lesions treated with SDF. For example, it has been investigated the changes of lesion depth in artificial dentine lesion after treatment with 38% SDF and 8 days of pH cycling regime. The results were compared to 10% NaF solution, 42% silver nitrate solution and deionised water (control). Smaller depth of lesion was noted in the lesion treated with SDF, and it was statistically significant when compared to the control and silver nitrate group (Mei et al., 2013b). However, the sample size was small ($n=6$) in each group. This method may be limited by artefacts produced in the image after x-ray exposure caused by the silver and iodide particles which are radiopaque (Öztürk et al., 2015, FDA, 2001).

Surface Microhardness (SMH) was not considered for one of the methods of analysis because the artificial dentine lesions were expected to be soft on the surface and not reliable with the Knoop hardness measurement as proposed by Magalhaes et al. (2009).

As stated in the literature review, cross-sectional micro hardness (CSMH) is one of the valuable tools in evaluating demineralisation and remineralisation process (Arends and Schuthof, 1980, Arends et al., 1980, Arends and Tenbosch, 1992, Magalhaes et al., 2009, Hara et al., 2003). The changes in mineral content of the carious teeth can be indirectly measured using the hardness testing. Featherstone et al. (1983) in their study, concluded that the cross-sectional microhardness as a comparative measure of hardness changes and could measure the mineral loss or gain directly after a demineralisation and potentially remineralisation process. Therefore, the Knoop hardness (KHN) parameter was considered as the main indicator for mineral loss and progression or regression in the present study. Also, it has been tested in the laboratory the feasibility of CSMH for analysing lesions treated with SDF prior to the main study.

In the current study, the dentine slabs in resin blocks were secured to a glass microscope slide and waited until the glue dried before any indentations were made to avoid any tilting or movement of the specimen. The indenter table was also standardised and secured, thereby controlling specimen stability and magnification.

Three rows of 15 indentations each were made, one at the central region of the dentine lesion, and the other two at 100 μm apart. The indentations were made at every 20 μm from the lesion surface until 300 μm deep. The mean hardness and standard deviation for each group according to the distance from the lesion surface were calculated. Several studies reported the arrested dentine hardness values in Knoop hardness ranges from 25 to 65 KHN (Hosoya et al., 2000, Banerjee et al., 1999, Pereira et al., 1998, Mäkinen et al., 1998). However, the microhardness values cannot be directly measured between different studies due to several factors. The preparation of the specimens and the load used for indentation can vary greatly from one study to another (Chu and Lo, 2008a). For example, Moron et al. (2013) used a load of 10 gram applied for 10 seconds in evaluating the cross-sectional microhardness of dentine lesions. Hara et al. (2003) reported no signs of crack or fractures in dentine lesions analysed with cross-sectional microhardness taken at 10 μm depth using 5 g load for 5 s of Knoop indenter. Another study by Marquezan et al. (2009) and Hosoya et al. (2000) used a static load of 25 g for 30 s on sound dentine and 10 g for 30 s on carious dentine. They claimed that this approach was taken to minimise fractures in the samples, and it was difficult to obtain a reliable indentation when using different loads for the same lesion before and after caries induction. Therefore, interpretation of the results should be done critically because different load indentation used. The Knoop indentation used for this study was a load of 10 gram for 5 seconds for all of the specimens showed reliable indentation and did not show any cracks or fractures of the outer edge of the lesions.

The vertical distance between each indentation was also differing from one study to another. In general, most of the studies that used cross-sectional microhardness analysis of dentine lesions have 10-30 μm gaps between indentations up to 500 μm of depth from the outer surface. Variability in the hardness of the dentine is also great due to differences in dentine substrate used,

lesion preparation methods and types of lesions analysed. Nevertheless, most studies that evaluated the effectiveness of SDF in terms of microhardness reported a significant increase in hardness after application of SDF when compared to non-treated lesions (Chu and Lo, 2008b, Chu and Lo, 2008a, Mei et al., 2013c)

Paired t-test was used to test the intra-examiner reliability showed no significant difference between the mean dentine Knoop hardness (KHN) for all untreated and treated samples ($p > 0.05$). This result represents excellent reliability.

6.7 Scanning Electron Microscopy (SEM)

A scanning electron microscope generates a magnified image of a specimen by scanning the surface with a focused beam of electrons. It has been widely used in dental caries study. The images from SEM analysis provides valuable information about the mechanism of carious lesion formation and of its repair. Another advantage of this technique is the ability to map certain elements on the lesion surface. SEM images of dentine lesions were analysed for SDF, SDF/KI and W groups and unable to provide the microstructure of the dentine lesions for FV group in present study.

6.8 Remineralising potential of Silver Diamine Fluoride (SDF) with or without potassium iodide (SDF/KI) and fluoride varnish (FV) on artificial dentine lesions *in vitro*

The main study investigated the effect of silver diamine fluoride (SDF), SDF followed by potassium iodide (SDF/KI) and fluoride varnish (FV) on artificial dentine caries *in vitro*. Deionised water (W) was used as a negative control. The outcome of the study reveals significant differences according to the parameter used.

Increase in dentine hardness in the artificial caries lesion suggests an increase in the mineral content and could be considered as a sign of remineralisation (Chu and Lo, 2008a). The study results demonstrated no evidence of remineralisation of the artificial dentine subsurface lesion after treatment with the distilled water

group (W), with a statistically significant demineralisation starting from level 60 μm up to 300 μm . No significant difference was seen at the first 40 μm depth into the lesion.

The SDF group showed statistically significant remineralisation between untreated and after treatment for the first 60 μm outer layer of the lesion. The beneficial effects of SDF on the artificial subsurface dentine lesion is only up to 100 μm . The lesion appeared to be softer after subjected to pH cycling challenge at 120 μm and above where statistically significant demineralisation can be seen at 200, 240, 260, 280 μm from lesion surface. A similar trend can also be found in SDF/KI and FV group.

The highest remineralisation can be seen in just underneath the lesion surface in SDF group, followed by SDF/KI and FV group. When comparing the difference in dentine hardness of groups after the acidic challenge, there was no statistical difference in change of dentine hardness at level 80 and 100 μm across all groups. Remineralisation in SDF and SDF/KI group was significantly higher than W group at almost all levels and FV group only at 20 μm . No significant in remineralisation difference in SDF compared to SDF/KI group and FV compared to SDF/KI group. There could be a significant difference between the dentine hardness of group SDF and SDF/KI. However, to test this hypothesis, it will require more specimens than we can access at this time. Furthermore, SDF/KI is indicated for the treatment of dentine hypersensitivity (Craig et al., 2012, Castillo et al., 2011), whilst caries arresting effect of SDF/KI has not yet established.

The pattern of decreasing hardness in deeper layers as observed in all groups suggested less mineral loss occurred in the outer layer of the artificial dentine subsurface lesion after subjected to the pH cycling regime. It seems possible that these results are due to the permeability of dentine to acids and larger contact areas with cariogenic acids compared to enamel (Buzalaf et al., 2011). For this reason, demineralisation occurs at relatively larger depth, whilst mineral deposition is confined to the outer layers (Buzalaf et al., 2011). In SDF, SDK/KI and FV group, the dentinal tubules are coated with a fluoride-rich mineral which makes the dentine relatively resistant to acid attacks. During the pH cycling regime, the acids bypass these relatively resistant layers, readily depositing

minerals and fluoride ions making the lesion moves deeper but broader at the surface layer. In such a way, the mineral gain is more predominant at the surface layer and mineral loss at the lesion front in dentine lesions (ten Cate et al., 1995). One could postulate that the little change in dentine hardness of the outer layer for Group W is due to the inhibition of demineralisation action by the buffering action of saliva throughout the acidic challenge.

These results corroborate the findings of a great deal of the previous work, where the outer 125 μm of the artificial dentine lesion is significantly harder after treatment with SDF in contrast to the W group (Chu et al., 2012). Although, Chu et al., (2012) used a very different model, exposing dentine blocks to bacteria over seven days. This means that they had no remineralisation component, compared to the pH-cycling experiment of like present study. A previous study also indicated that dentine hardness of the outermost layer of dentine caries lesion treated with SDF was greater compared to active carious lesions (Chu and Lo, 2008a). However, these studies found no difference in dentine hardness at a level deeper in the lesion. This is probably since the study consisted of exfoliated treated and untreated teeth compared to the controlled environment of the current study. Also, the present study provides a baseline to compare the progression of the lesion over the course of the study allowing a more detailed analysis of lesion progression.

In all treatment groups, there was an increase hardness of the dentine closer to the surface, except W group. The observed decrease hardness deeper in and below the lesion could partly be due to the solubility of the dentine. The previously demineralised outer layer will have already lost their most soluble components, whereas deeper in the lesion, these more soluble components will still be present. The observation that the lesions are simultaneously undergoing both remineralisation and demineralisation is consistent with studies into the chemistry of lesion formation (Robinson et al., 1995, Robinson et al., 2000). At the lesion front (deepest part of the lesion), dissolution of the dentine, especially the more soluble carbonate and magnesium substituted crystals occurs. While closer to the lesion surface, the more stable crystals and the presence of fluoride along with the minerals dissolved from deeper into the dentine resulting in net remineralisation.

FV group showed significant remineralisation in the first 40 µm of the dentine lesion in the untreated. However, the mean difference of KHN between FV and W group was not significant. Furthermore, the mean difference of KHN between SDF and SDF/KI and FV and groups were statistically significant. This observation could be due to the loss of CaF₂ precipitation being washed away throughout the experiment and lack of silver ions, which contributes to the lesion hardness (Lou et al., 2011). These results seem to be consistent with other research which found the superiority of SDF products over topical fluoride treatment in preventing dentine caries clinically (Chu et al., 2002, Yee et al., 2009, Llodra et al., 2005), in *ex vivo* (Mei et al., 2014) and *in vitro* studies (Mei et al., 2013b, Yu et al., 2018, Zhao et al., 2017). However, the current evidence on the benefits of SDF application for caries prevention is of low-quality due to high risk of bias (Oliveira et al., 2019, Gao et al., 2016, Burgess and Vaghela, 2018). Hence, more clinical trials were suggested to establish robust and high-quality evidence on the clinical efficacy of SDF applications for caries prevention.

Nevertheless, fluoride varnishes are more widely studied compared to SDF products and is indeed effective in preventing the progression of carious lesions (Gao et al., 2016, Marinho et al., 2013, Twetman et al., 2003, Marinho et al., 2003d, Marinho et al., 2003b, Marinho et al., 2003c, Marinho et al., 2003a).

In the current study, SEM images were captured to assess the morphological changes of artificial subsurface dentine lesion after different topical fluoride therapy over a acidic challenge. Group W showed enlargement of dentinal tubules over the course of acidic challenge giving an 'etched' appearance of the artificial subsurface lesions. The dentinal tubules also appeared larger and well-defined when compared to SDF and SDF/KI groups. No precipitates formed on the lesion surface. Only some of the dentinal tubules were blocked.

When observed under SEM, a layer of protective materials can be seen formed over the artificial subsurface dentine lesions with some exposed areas in the SDF group only. A possible explanation for this might be that calcium fluoride (CaF₂)-like globules were formed after exposure to 40% SDF solution, which contains 120,000 ppm fluoride (Gotjamanos and Afonso, 1997). The CaF₂ globules served

as an important fluoride reservoir for the tooth structure and played an important role in promoting remineralisation. It also has been suggested that silver phosphate is formed and precipitated when a carious lesion is treated with SDF (Chu and Lo, 2008b). An earlier study by Yu et al. (2001) reported the formation of a less or nearly insoluble layer of CaF_2 , silver fluoride (AgF), silver phosphate (Ag_3PO_4) and silver protein on dentine surface after treatment with SDF. Some of the silver salt formed after application could have been lost throughout the course of 28 days of pH-cycling, which explains the lost part of the protective layer in the SDF and SDF/KI groups. This is probably due to the high solubility of the CaF_2 precipitation being easily washed away by deionised water in *in vitro* studies (Lou et al., 2011). It can be thus suggested that the cause lower remineralisation in FV group and no remineralisation in W group is due to this reason.

Upon higher magnification, there was an accumulation of spherical particles partially blocking the dentine tubules and peritubular areas in the artificial dentine lesions treated with SDF and SDF/KI group when observed under SEM. Elemental analysis under SEM of SDF group showed silver (Ag) particles mapped in the corresponding protective layer areas, whilst fluoride (F) mapped more densely on the Ag areas and distributed all over the lesion surface. The presence of these elements after a period of acidic challenge can be regarded as an evidence of remineralisation of the artificial subsurface dentine lesions and probably explains the higher dentine hardness after treatment with SDF group when compared to SDF/KI, FV and W groups.

The findings for the SDF products supports evidence from previous observations done by Mei et al. (2013b) and Zhao et al. (2017). The accumulation of granular particles in the dentinal tubules after exposure to a remineralising agent may have an important role in increasing the hardness of the dentine surface and its resistance to further mineral loss. Furthermore, the partial blocking of the dentinal tubules could also be responsible in reducing dentine hypersensitivity and preventing caries progression (Arends et al., 1989, Arends et al., 1997, Mei et al., 2013b). Hiraishi et al. (2010) in their *in vitro* study showed SDF penetration into in the subsurface dentinal tubules up to 40 μm in depth, whereas Willershausen et al. (2015) detected silver particles only at 20 μm . These silver deposits could

possibly act as a reservoir of silver ions where they contribute to its antibacterial properties and increase in dentine hardness after treatment with SDF products. However, the observation of the precipitation layer as seen in SDF group was not found in other studies.

One of the most significant findings that emerged from this study was that, in FV group, the hardness of dentine lesion was higher than the untreated lesions up to 80 μm depth, but the values were much softer when compared to SDF and SDF/KI group. It is possible to hypothesise that the increase of dentine hardness after application of the experimental materials is due to infiltrations of the fluoride and silver ions into the dentinal tubules. The dentine slabs in this present study were prepared in such a way to ensure the direction of dentine tubules are perpendicular to the lesion surface as much as possible because the direction of the dentinal tubules plays an important role in the depth of penetration of topical fluoride products. Dentinal tubules that are perpendicular to the lesion surface infiltrates deeper into the lesion compared to those that are parallel to the outer surface. Arends et al. (1997) found evidence of fluoride varnishes could penetrate into carious lesions up to 85 μm in depth, which is deeper than for lesions treated with SDF. This explains the anti-caries effect and prevention of hypersensitivity of fluoride varnishes by penetration into the dentine and 'seals' the tubules completely or partly. The differences in these findings were mainly due to differences in materials used, methodology and hypotheses.

The results of this investigation showed that the remineralisation by the SDF products and fluoride varnish is only evident at the outer layer of the dentine lesions. It is possible that the lesion will continue deeper and much softer if the acidic challenge is longer and no additional fluoride is supplied to the lesion. Therefore, this finding supports the periodic application topical fluoride to prevent progression of caries in clinical situations as recommended in multiple reviews (England, 2017, Marinho et al., 2013, Lussi et al., 2012, Marinho et al., 2003d, Poulsen, 2009) and guidelines (Crystal et al., 2017, England, 2017).

It is also important to bear in mind that this is an *in vitro* study, done in a controlled laboratory environment, and the substrate of bovine dentine. There were no other variables such as oral bacteria, tooth cleansing action by masticatory muscles,

toothbrushing as found in *in vivo*, *in situ* and clinical trials. Therefore, a further study, which take these variables into account, will need to be undertaken.

6.9 Suggestions for future research

These findings raise intriguing questions regarding the nature and extent of the effectiveness of professionally applied topical fluorides. The protection of the outer layers but not in the deeper levels of the dentine lesions after treatment with the experimental materials warrants more studies to be carried out in order to validate these findings.

The pH cycling model used in this present is only 28 days. In future investigations, it might be possible to use a longer period of pH cycling with repeated fluoride application. Increasing the sample size may show more differences between groups.

Cross sectional microhardness was used to analyse the mineral content of the artificial subsurface dentine lesions. Additional analysis using micro-CT scan would provide more information regarding the lesion behaviour before and after treatment.

The images of the cross-sectioned surface were not captured in this present study. It would be interesting to analyse the lesion at a higher magnification and depth of the penetration of the SDF into the dentinal tubules in the lesion and compare between groups.

It would be interesting to see future work with the same protocol using human dentine substrate, either primary or permanent teeth as opposed to the bovine samples of the current method. Future *in situ* and high-quality *in vivo* studies investigating the remineralisation potential of different topical fluoride products may provide more clinical relevance of its use.

6.10 Outcome of the null hypothesis (II)

The null hypothesis “There is no difference in the effect of Silver Diamine Fluoride (SDF), SDF with potassium iodide (SDF/KI) and Fluoride varnish (FV) on progression/ regression of dentine artificial subsurface lesions under pH-cycling regime *in vitro*.” should be rejected as significant differences were found in remineralisation of dentine lesions between the test groups.

Chapter 7 Conclusion

From the results of the present study, it can be concluded that:

1. A statistically significant remineralisation in the outer layers of the artificial subsurface dentine lesions up to 100 μm in comparison with the untreated was found in all groups except the W group.
2. SDF has the highest remineralising potential on artificial subsurface dentine lesions *in vitro*, followed by SDF/KI then FV.
3. SDF has the ability to minimise lesion progression in the deeper depth compared to other groups. However, remineralisation only confined to the outer dentine lesion.
4. Elemental analysis confirms the presence of silver particles and fluoride remains on the lesion surface after the acidic challenge.

In spite of its limitations, the study certainly adds to our understanding of the mechanism of action of SDF products in dentine lesions.

References

- ABDULLAH, A. 2009. *In vitro and in situ to investigate the erosion of human dental tissues*. PhD thesis, University of Leeds.
- AL-KANDARI, J. J. A. M. A. 2017. *Evaluation of the remineralisation of enamel by different formulations and concentrations of fluoride toothpastes in vitro*. D.Paed.Dent thesis, University of Leeds.
- AL-MULLAHI, A. M. & TOUMBA, K. J. 2010. Effect of Slow-Release Fluoride Devices and Casein Phosphopeptide/Amorphous Calcium Phosphate Nanocomplexes on Enamel Remineralization in vitro. *Caries Research*, 44, 364-371.
- AMAECHI, B. T., HIGHAM, S. M. & EDGAR, W. M. 1998. Factors affecting the development of carious lesions in bovine teeth in vitro. *Archives of Oral Biology*, 43, 619-628.
- ARENDS, J., CHRISTOFFERSEN, J., RUBEN, J. & JONGEBLOED, W. L. 1989. Remineralization of bovine dentine in vitro. The influence of the F content in solution on mineral distribution. *Caries Res*, 23, 309-14.
- ARENDS, J., DUSCHNER, H. & RUBEN, J. L. 1997. Penetration of varnishes into demineralized root dentine in vitro. *Caries Research*, 31, 201-205.
- ARENDS, J. & SCHUTHOF, J. 1980. Microhardness and lesion depth studies of artificial caries lesions - a comparison of gelatin and hec based systems. *Journal De Biologie Buccale*, 8, 175-181.
- ARENDS, J., SCHUTHOF, J. & JONGEBLOED, W. G. 1980. Lesion depth and microhardness indentations on artificial white spot lesions. *Caries Research*, 14, 190-195.
- ARENDS, J. & TENBOSCH, J. J. 1992. Demineralization and remineralization evaluation techniques. *Journal of Dental Research*, 71, 924-928.
- AXELSSON, P. 2004. *Preventive materials, methods, and programs*, Chicago London, Quintessence Publications.
- BANERJEE, A., SHERRIFF, M., KIDD, E. A. M. & WATSON, T. F. 1999. A confocal microscopic study relating the autofluorescence of carious dentine to its microhardness. *British Dental Journal*, 187, 206-210.
- BURGESS, J. O. & VAGHELA, P. M. 2018. Silver Diamine Fluoride: A Successful Anticariogenic Solution with Limits. *Advances in Dental Research*, 29, 131-134.
- BURT, B. A. 1992. THE CHANGING PATTERNS OF SYSTEMIC FLUORIDE INTAKE. *Journal of Dental Research*, 71, 1228-1237.
- BUZALAF, M. A., PESSAN, J. P., HONORIO, H. M. & TEN CATE, J. M. 2011. Mechanisms of action of fluoride for caries control. In: BUZALAF, M. A. R. (ed.) *Fluoride and the Oral Environment*. Basel: Karger.
- BUZALAF, M. A. R., HANNAS, A. R., MAGALHAES, A. C., RIOS, D., HONORIO, H. M. & DELBEM, A. C. B. 2010. pH-cycling models for in vitro evaluation of the efficacy of fluoridated dentifrices for caries control: strengths and limitations. *Journal of Applied Oral Science*, 18, 316-334.
- CASTILLO, J. L., RIVERA, S., APARICIO, T., LAZO, R., AW, T. C., MANCL, L. L. & MILGROM, P. 2011. The short-term effects of diammine silver fluoride on tooth sensitivity: a randomized controlled trial. *J Dent Res*, 90, 203-8.
- CHONG, L. Y., CLARKSON, J. E., DOBBYN-ROSS, L. & BHAKTA, S. 2018. Slow-release fluoride devices for the control of dental decay. *Cochrane Database of Systematic Reviews*, 28.

- CHU, C., LO, E. & LIN, H. 2002. Effectiveness of silver diamine fluoride and sodium fluoride varnish in arresting dentin caries in Chinese pre-school children. *Journal of Dental Research*, 81, 767-770.
- CHU, C. H. & LO, E. C. 2008a. Microhardness of dentine in primary teeth after topical fluoride applications. *J Dent*, 36, 387-91.
- CHU, C. H. & LO, E. C. 2008b. Promoting caries arrest in children with silver diamine fluoride: a review. *Oral Health & Preventive Dentistry*, 6, 315-321.
- CHU, C. H., MEI, L., SENEVIRATNE, C. J. & LO, E. C. 2012. Effects of silver diamine fluoride on dentine carious lesions induced by *Streptococcus mutans* and *Actinomyces naeslundii* biofilms. *Int J Paediatr Dent*, 22, 2-10.
- CRAIG, G. G., KNIGHT, G. M. & MCINTYRE, J. M. 2012. Clinical evaluation of diamine silver fluoride/potassium iodide as a dentine desensitizing agent. A pilot study. *Australian Dental Journal*, 57, 308-311.
- CRAIG, G. G., POWELL, K. R. & PRICE, C. A. 2013. Clinical evaluation of a modified silver fluoride application technique designed to facilitate lesion assessment in outreach programs. *Bmc Oral Health*, 13, 6.
- CRYSTAL, Y. O., MARGHALANI, A. A., URELES, S. D., WRIGHT, J. T., SULYANTO, R., DIVARIS, K., FONTANA, M. & GRAHAM, L. 2017. Use of Silver Diamine Fluoride for Dental Caries Management in Children and Adolescents, Including Those with Special Health Care Needs. *Pediatric Dentistry*, 39, E135-E145.
- DE JOSSELIN, E., SUNDSTROM, F., WESTERLING, H., TRANAEUS, S., TEN BOSCH, J. J. & ANGMAR-MANSSON, B. 1995. A new method for in vivo quantification of changes in initial enamel caries with laser fluorescence. *Caries Res*, 29, 2-7.
- DEAN, H. T., ARNOLD, J., F A, JAY, P. & KNUTSON, J. W. 1950. Studies on mass control of dental caries through fluoridation of the public water supply. *Public Health Reports (1896-1970)*, 65, 1403-1408.
- DEERY, C. & TOUMBA, K. J. 2008. Diagnosis and prevention of dental caries. In: WELBURY, R. R., DUGGAL, M. S. & HOSEY, M. (eds.) *Paediatric Dentistry*. Third ed.: Oxford University Press.
- DRIESSEN, F. C. M. & VERBEECK, R. M. H. 1981. The probable phase composition of the minerals in sound enamel and dentin. *Bulletin des Societies Chimiques Belges*, 91, 573-596.
- DUFFIN, S. 2012. Back to the future: the medical management of caries introduction. *J Calif Dent Assoc*, 40, 909.
- DUGGAL, M. S., TOUMBA, K. J., AMAECHI, B. T., KOWASH, M. B. & HIGHAM, S. M. 2001. Enamel demineralization in situ with various frequencies of carbohydrate consumption with and without fluoride toothpaste. *Journal of Dental Research*, 80, 1721-1724.
- EL TANTAWI, M., FOLAYAN, M. O., MEHAINA, M., VUKOVIC, A., CASTILLO, J. L., GAFFAR, B. O., ARHEIAM, A., AL-BATAYNEH, O. B., KEMOLI, A. M., SCHROTH, R. J. & LEE, G. H. M. 2018. Prevalence and Data Availability of Early Childhood Caries in 93 United Nations Countries, 2007-2017. *American Journal of Public Health*, 108, 1066-1072.
- ENGLAND, P. H. 2017. Delivering better oral health: an evidence-based toolkit for prevention. In: ENGLAND, P. H. (ed.). London.
- ESPELID, I. 2009. Caries preventive effect of fluoride in milk, salt and tablets: a literature review. *European archives of paediatric dentistry : official journal of the European Academy of Paediatric Dentistry*, 10, 149-56.

- FDA 2001. Guidance Potassium Iodide as a Thyroid Blocking Agent in Radiation Emergencies
 . In: SERVICES, U. S. D. O. H. A. H. (ed.). United States of America.
- FEATHERSTONE, J. D. 1999. Prevention and reversal of dental caries: role of low level fluoride. *Community Dent Oral Epidemiology*, 27, 31-40.
- FEATHERSTONE, J. D. 2000. The science and practice of caries prevention. *Journal of American Dental Association*, 131, 887-99.
- FEATHERSTONE, J. D. 2004. The Continuum of Dental Caries--Evidence for a Dynamic Disease Process. *Journal of Dental Research*, 83, C39-C42.
- FEATHERSTONE, J. D., GLENA, R., SHARIATI, M. & SHIELDS, C. P. 1990. Dependence of in vitro demineralization of apatite and remineralization of dental enamel on fluoride concentration. *Journal of dental research*, 69 Spec No, 620-5; discussion 634-6.
- FEATHERSTONE, J. D. & MELLBERG, J. R. 1981. Relative rates of progress of artificial carious lesions in bovine, ovine and human enamel. *Caries Res*, 15, 109-14.
- FEATHERSTONE, J. D., TENCATE, J. M., SHARIATI, M. & ARENDS, J. 1983. Comparison of artificial caries-like lesions by quantitative microradiography and microhardness profiles. *Caries Research*, 17, 385-391.
- FEATHERSTONE, J. D. & ZERO, D. T. 1992. An insitu model for simultaneous assessment of inhibition of demineralization and enhancement of remineralization. *Journal of Dental Research*, 71, 804-810.
- FEJERSKOV, O. & KIDD, E. A. M. 2008. *Dental caries: the disease and its clinical management*, Oxford, Blackwell Munksgaard.
- FONTANA, M., YOUNG, D. A., WOLFF, M. F., PITTS, N. B. & LONGBOTTOM, C. 2010. Defining Dental Caries for 2010 and Beyond. *Dental Clinics of North America*, 54, 423-440.
- GAO, S. S., ZHANG, S., MEI, M. L., LO, E. C.-M. & CHU, C.-H. 2016. Caries remineralisation and arresting effect in children by professionally applied fluoride treatment - a systematic review. *Bmc Oral Health*, 16.
- GMUR, R., GIERTSEN, E., VAN DER VEEN, M. H., DE JONG, E. D., TEN CATE, J. M. & GUGGENHEIM, B. 2006. In vitro quantitative light-induced fluorescence to measure changes in enamel mineralization. *Clinical Oral Investigations*, 10, 187-195.
- GOMEZ, J. 2015. Detection and diagnosis of the early caries lesion. *Bmc Oral Health*, 15, 7.
- GOTJAMANOS, T. & AFONSO, F. 1997. Unacceptably high levels of fluoride in commercial preparations of silver fluoride. *Australian Dental Journal* 42, 52-53.
- HAMAMA, H. H., YIU, C. K. & BURROW, M. F. 2015. Effect of silver diamine fluoride and potassium iodide on residual bacteria in dentinal tubules. *Australian Dental Journal*, 60, 80-87.
- HARA, A. T., QUEIROZ, C. S., LEME, A. F., SERRA, M. C. & CURY, J. A. 2003. Caries progression and inhibition in human and bovine root dentine in situ. *Caries Research*, 37, 339-344.
- HAZELRIGG, C., DEAN, J. & FONTANA, M. 2003. Fluoride varnish concentration gradient and its effect on enamel demineralization. *Pediatr Dent*, 25, 119-26.
- HEIFETZ, S. B. & HOROWITZ, H. S. 1986. AMOUNTS OF FLUORIDE IN SELF-ADMINISTERED DENTAL PRODUCTS - SAFETY CONSIDERATIONS FOR CHILDREN. *Pediatrics*, 77, 876-882.

- HIRAISHI, N., YIU, C. K., KING, N. M., TAGAMI, J. & TAY, F. R. 2010. Antimicrobial efficacy of 3.8% silver diamine fluoride and its effect on root dentin. *J Endod*, 36, 1026-9.
- HODGE, H. C. & SMITH, F. A. 1965. Biological effects of inorganic fluorides. In: SIMON, J. H. (ed.) *Fluorine Chemistry*. New York: Academic Press.
- HORAK, D., CERVINKA, M. & PUZA, V. 1998. Radiopaque poly(2-hydroxyethyl methacrylate) particles containing silver iodide complexes tested on cell culture. *Biomaterials*, 19, 1303-1307.
- HORST, J. A., ELLENKIOTIS, H. & MILGROM, P. L. 2016. UCSF Protocol for Caries Arrest Using Silver Diamine Fluoride: Rationale, Indications and Consent. *Journal of the California Dental Association*, 44, 16-28.
- HOSOYA, Y., MARSHALL, S. J., WATANABE, L. G. & MARSHALL, G. W. 2000. Microhardness of carious deciduous dentin. *Operative Dentistry*, 25, 81-89.
- HUMPHREY, S. P. & WILLIAMSON, R. T. 2001. A review of saliva: Normal composition, flow, and function. *The Journal of Prosthetic Dentistry*, 85, 162-169.
- IHEOZOR-EJIOFOR, Z., WORTHINGTON, H. V., WALSH, T., O'MALLEY, L., CLARKSON, J. E., MACEY, R., ALAM, R., TUGWELL, P., WELCH, V. & GLENNY, A. M. 2015. Water fluoridation for the prevention of dental caries. *Cochrane Database of Systematic Reviews*, 276.
- ISSA, A. 2004. *The effect of intrinsic and extrinsic sugars on enamel demineralisation and plaque pH as determined using intraoral cariogenicity tests and plaque pH telemetry*. PhD Thesis, University of Leeds.
- JALLAD, M., ZERO, D., ECKERT, G. & ZANDONA, A. F. 2015. In vitro Detection of Occlusal Caries on Permanent Teeth by a Visual, Light-Induced Fluorescence and Photothermal Radiometry and Modulated Luminescence Methods. *Caries Research*, 49, 523-530.
- JASIM, S., WENGER, D. & WERMERS, R. A. 2018. SKELETAL FLUOROSIS RELATED TO HABITUAL TEA CONSUMPTION: LONG-TERM FOLLOW-UP AFTER REDUCTION AND DISCONTINUATION OF TEA. *AACE Clinical Case Reports*, 4, e98-e103.
- KLASEN, H. J. 2000. Historical review of the use of silver in the treatment of burns. I. Early uses. *Burns*, 26, 117-130.
- KNIGHT, G. M., MCINTYRE, J. M., CRAIG, G. G. & MULYANI 2006a. Ion uptake into demineralized dentine from glass ionomer cement following pretreatment with silver fluoride and potassium iodide. *Australian Dental Journal*, 51, 237-241.
- KNIGHT, G. M., MCINTYRE, J. M., CRAIG, G. G., MULYANI, ZILM, P. S. & GULLY, N. J. 2005. An in vitro model to measure the effect of a silver fluoride and potassium iodide treatment on the permeability of demineralized dentine to *Streptococcus mutans*. *Australian Dental Journal*, 50, 242-245.
- KNIGHT, G. M., MCINTYRE, J. M., CRAIG, G. G., MULYANI, ZILM, P. S. & GULLY, N. J. 2007. Differences between normal and demineralized dentine pretreated with silver fluoride and potassium iodide after an in vitro challenge by *Streptococcus mutans*. *Australian Dental Journal*, 52, 16-21.
- KNIGHT, G. M., MCINTYRE, J. M. & MULYANI 2006b. The effect of silver fluoride and potassium iodide on the bond strength of auto cure glass ionomer cement to dentine. *Australian Dental Journal*, 51, 42-45.

- KOULOURLIDES, T. & VOLKER, J. F. 1964. Fortification of enamel by extracts of silicate cements + fluoride-containing solutions. *Journal of Dental Research*, 43, 865-&.
- KUEHNISCH, J., IFLAND, S., TRANAEUS, S., ANGMAR-MANSSON, B., HICKEL, R., STOSSER, L. & HEINRICH-WELTZIEN, R. 2006. Establishing quantitative light-induced fluorescence cut-offs for the detection of occlusal dentine lesions. *European Journal of Oral Sciences*, 114, 483-488.
- LAGERLÖF, F. 1994. Caries-Protective Factors in Saliva. *Advances in Dental Research*, 8, 229-238.
- LARMAS, M. 1986. Response of pulpo-dentinal complex to caries attack. *Proc Finn Dent Soc* 82, 298-304.
- LEGEROS, R. Z. 1991. Calcium phosphates in oral biology and medicine. *Monographs in oral science*, 15.
- LLODRA, J. C., RODRIGUEZ, A., FERRER, B., MENARDIA, V., RAMOS, T. & MORATO, M. 2005. Efficacy of Silver Diamine Fluoride for Caries Reduction in Primary Teeth and First Permanent Molars of Schoolchildren: 36-month Clinical Trial. *Journal of Dental Research*, 84, 721-724.
- LO, E. C. M., ZHI, Q. H. & ITTHAGARUN, A. 2010. Comparing two quantitative methods for studying remineralization of artificial caries. *Journal of Dentistry*, 38, 352-359.
- LOESCHE, W. 1986. Role of Streptococcus mutans in Human Dental Decay. *Microbiological Reviews*, 50, 352-80.
- LONGBOTTOM, C. L., HUYSMANS, M. C., PITTS, N. B. & FONTANA, M. 2009. Glossary of key terms. *Monographs in oral science*, 21, 209-216.
- LOU, Y. L., BOTELHO, M. G. & DARVELL, B. W. 2011. Reaction of silver diamine fluoride with hydroxyapatite and protein. *Journal of Dentistry*, 39, 612-618.
- LUSSI, A., HELLWIG, E. & KLIMEK, J. 2012. Fluorides - mode of action and recommendations for use. *Schweizer Monatsschrift für Zahnmedizin = Revue mensuelle suisse d'odonto-stomatologie = Rivista mensile svizzera di odontologia e stomatologia*, 122, 1030-42.
- MAGALHAES, A. C., MORON, B. M., COMAR, L. P., WIEGAND, A., BUCHALLA, W. & BUZALAF, M. A. R. 2009. Comparison of Cross-Sectional Hardness and Transverse Microradiography of Artificial Carious Enamel Lesions Induced by Different Demineralising Solutions and Gels. *Caries Research*, 43, 474-483.
- MÄKINEN, K. K., CHIEGO, D. J. J., ALLEN, P., BENNETT, C., ISOTUPA, K. P., TIEKSO, J. & MÄKINEN, P. L. 1998. Physical, chemical, and histological changes in dentin caries lesions of primary teeth induced by regular use of polyol chewing gums. *Acta Odontol Scand.*, 56, 148-56.
- MALINOWSKI, M. 2010. *In situ studies with fluoridated milk for the remineralisation of dental enamel*. PhD thesis, University of Leeds.
- MARINHO, V. C., HIGGINS, J. P., SHEIHAM, A. & LOGAN, S. 2003a. Fluoride toothpastes for preventing dental caries in children and adolescents. *Cochrane Database Syst Rev*, CD002278.
- MARINHO, V. C. C., HIGGINS, J. P. T., LOGAN, S. & SHEIHAM, A. 2003b. Fluoride mouthrinses for preventing dental caries in children and adolescents. *The Cochrane database of systematic reviews*, CD002284-CD002284.
- MARINHO, V. C. C., HIGGINS, J. P. T., LOGAN, S. & SHEIHAM, A. 2003c. Systematic review of controlled trials on the effectiveness of fluoride gels

- for the prevention of dental caries in children. *Journal of dental education*, 67, 448-58.
- MARINHO, V. C. C., HIGGINS, J. P. T., LOGAN, S. & SHEIHAM, A. 2003d. Topical fluoride (toothpastes, mouthrinses, gels or varnishes) for preventing dental caries in children and adolescents. *The Cochrane database of systematic reviews*, CD002782-CD002782.
- MARINHO, V. C. C., WORTHINGTON, H. V., WALSH, T. & CHONG, L. Y. 2015. Fluoride gels for preventing dental caries in children and adolescents. *Cochrane Database of Systematic Reviews*, 114.
- MARINHO, V. C. C., WORTHINGTON, H. V., WALSH, T. & CLARKSON, J. E. 2013. Fluoride varnishes for preventing dental caries in children and adolescents. *Cochrane Database of Systematic Reviews*, 95.
- MARKOWITZ, K. & PASHLEY, D. H. 2008. Discovering new treatments for sensitive teeth: the long path from biology to therapy. *Journal of Oral Rehabilitation*, 35, 300-315.
- MARQUEZAN, M., CORRÊA, F. N. P., SANABE, M. E., RODRIGUES FILHO, L. E., HEBLING, J., GUEDES-PINTO, A. C. & MENDES, F. M. 2009. Artificial methods of dentine caries induction: A hardness and morphological comparative study. 54, 1111-1117.
- MARTINDALE, S. S. 2009. *the complete drug reference*, United Kingdom, Pharmaceutical Press.
- MCDONAGH, M. S., WHITING, P. F., WILSON, P. M., SUTTON, A. J., CHESTNUTT, I., COOPER, J., MISSO, K., BRADLEY, M., TREASURE, E. & KLEIJNEN, J. 2000. Systematic review of water fluoridation. *Bmj*, 321, 855-859.
- MEI, M. L., CHU, C. H., LO, E. C. M. & SAMARANAYAKE, L. P. 2013a. Fluoride and silver concentrations of silver diammine fluoride solutions for dental use. *International Journal of Paediatric Dentistry*, 23, 279-285.
- MEI, M. L., ITO, L., CAO, Y., LI, Q. L., LO, E. C. M. & CHU, C. H. 2013b. Inhibitory effect of silver diamine fluoride on dentine demineralisation and collagen degradation. *Journal of Dentistry*, 41, 809-817.
- MEI, M. L., ITO, L., CAO, Y., LO, E. C. M., LI, Q. L. & CHU, C. H. 2014. An ex vivo study of arrested primary teeth caries with silver diamine fluoride therapy. *Journal of Dentistry*, 42, 395-402.
- MEI, M. L., LI, Q. L., CHU, C. H., LO, E. C. M. & SAMARANAYAKE, L. P. 2013c. Antibacterial effects of silver diamine fluoride on multi-species cariogenic biofilm on caries. *Annals of Clinical Microbiology and Antimicrobials*, 12, 7.
- MEI, M. L., LI, Q. L., CHU, C. H., YIU, C. K. Y. & LO, E. C. M. 2012. The inhibitory effects of silver diamine fluoride at different concentrations on matrix metalloproteinases. *Dental Materials*, 28, 903-908.
- MELLBERG, J. 1992a. Hard-tissue substrates for evaluation of cariogenic and anti-cariogenic activity in situ. *Journal of dental research*, 71, 913-919.
- MELLBERG, J. R. 1992b. HARD-TISSUE SUBSTRATES FOR EVALUATION OF CARIOGENIC AND ANTI-CARIOGENIC ACTIVITY INSITU. *Journal of Dental Research*, 71, 913-919.
- MORON, B. M., COMAR, L. P., WIEGAND, A., BUCHALLA, W., YU, H., BUZALAF, M. A. R. & MAGALHAES, A. C. 2013. Different Protocols to Produce Artificial Dentine Carious Lesions in vitro and in situ: Hardness and Mineral Content Correlation. *Caries Research*, 47, 162-170.
- NANCI, A. & TEN CATE, A. R. 2013. *Ten Cate's oral histology : development, structure, and function*, Elsevier.

- NHMRC 2007. A systematic review of the efficacy and safety of fluoridation. Canberra.
- OLIVEIRA, B. H., RAJENDRA, A., VEITZ-KEENAN, A. & NIEDERMAN, R. 2019. The Effect of Silver Diamine Fluoride in Preventing Caries in the Primary Dentition: A Systematic Review and Meta-Analysis. *Caries Research*, 53, 24-32.
- OPINYA, G. N. & IMALINGAT, B. 1991. Skeletal and dental fluorosis: two case reports. *East African Medical Journal*, 68.
- ORTIZ-RUIZ, A. J., TERUEL-FERNANDEZ, J. D., ALCOLEA-RUBIO, L. A., HERNANDEZ-FERNANDEZ, A., MARTINEZ-BENEYTO, Y. & GISPERT-GUIRADO, F. 2018. Structural differences in enamel and dentin in human, bovine, porcine, and ovine teeth. *Annals of Anatomy-Anatomischer Anzeiger*, 218, 7-17.
- ÖZOK, A. R., WU, M. K., TEN CATE, J. M. & WESSELINK, P. R. 2004. Effect of Dentinal Fluid Composition on Dentin Demineralization in vitro. *Journal of Dental Research*, 83, 849-853.
- ÖZTÜRK, F., ERSÖZ, M., ÖZTÜRK, S. A., HATUNOĞLU, E. & MALKOÇ, S. 2015. Micro-CT evaluation of microleakage under orthodontic ceramic brackets bonded with different bonding techniques and adhesives *European Journal of Orthodontics*, 38, 163-169.
- PENG, J. J. Y., BOTELHO, M. G. & MATINLINNA, J. P. 2012. Silver compounds used in dentistry for caries management: A review. *Journal of Dentistry*, 40, 531-541.
- PEREIRA, P. N., INOKOSHI, S., YAMADA, T. & TAGAMI, J. 1998. Microhardness of in vitro caries inhibition zone adjacent to conventional and resin-modified glass ionomer cement. *Dental materials*, 14, 179-185.
- PESSAN, J. P., TOUMBA, K. J. & BUZALAF, M. A. 2011. Topical use of fluorides for caries control. In: BUZALAF, M. A. (ed.) *Fluoride and the Oral Environment*. Basel: Karger.
- PETERSEN, P. E. & OGAWA, H. 2016. Prevention of dental caries through the use of fluoride - the WHO approach. *Community Dental Health*, 33, 66-68.
- PETERSSON, H. G. & BRATTHALL, D. 1996. The caries decline: A review of reviews. *European Journal of Oral Sciences*, 104, 436-443.
- PONTES, L. R. A., NOVAES, T. F., MORO, B. L. P., BRAGA, M. M. & MENDES, F. M. 2017. Clinical performance of fluorescence-based methods for detection of occlusal caries lesions in primary teeth. *Brazilian oral research*, 31, e91-e91.
- POULSEN, S. 2009. Fluoride-containing gels, mouth rinses and varnishes: an update of evidence of efficacy. *European archives of paediatric dentistry : official journal of the European Academy of Paediatric Dentistry*, 10, 157-61.
- PRESTON, K., HIGHAM, S. & SMITH, P. 2007. The efficacy of techniques for the disinfection of artificial sub-surface dentinal caries lesions and their effect on demineralization and remineralization in vitro. *Journal of dentistry*, 35, 490-495.
- PRETTY, I. A. 2006. Caries detection and diagnosis: Novel technologies. *Journal of Dentistry*, 34, 727-739.
- ROBINSON, C. 1995. The Chemistry of Dental Caries. In: ROBINSON, C., KIRKHAM, J. & SHORE, R. C. (eds.) *Dental enamel: Formation to destruction*. CRC Press.

- ROBINSON, C., KIRKHAM, J. & SHORE, R. 1995. Demineralisation and remineralisation of enamel. *In: ROBINSON, C., KIRKHAM, J. & SHORE, R. (eds.) Dental enamel: formation to destruction.* CRC Press, Inc.
- ROBINSON, C., SHORE, R., BROOKES, S. J., STRAFFORD, S. M., WOOD, S. R. & KIRKHAM, J. 2000. The Chemistry of Enamel Caries. *Critical Reviews in Oral Biology & Medicine*, 11, 481-495.
- RODRIGUES, E., DELBEM, A. C. B., PEDRINI, D. & OLIVEIRA, M. S. R. 2008. pH-cycling Model to Verify the Efficacy of Fluoride-releasing Materials in Enamel Demineralization. *Operative Dentistry*, 33, 658-665.
- ROOS, J., DUMOLARD, A., BOURGET, S., GRANGE, L., ROUSSEAU, A., GAUDIN, P., CALOP, J. & JUVIN, R. 2005. Osteofluorosis caused by excess use of toothpaste. *La Presse Médicale*, 34, 1518-1520.
- ROSENBLATT, A., STAMFORD, T. C. & NIEDERMAN, R. 2009. Silver diamine fluoride: a caries "silver-fluoride bullet". *J Dent Res*, 88, 116-25.
- RUEGGEBERG, F. A. 1991. Substrate for adhesion testing to tooth structure - review of the literature. *Dental Materials*, 7, 2-10.
- SAMPAIO, F. C. & LEVY, S. M. 2011. Systemic Fluoride. *In: BUZALAF, M. A. R. (ed.) Fluoride and the Oral Environment.* Karger, Postfach, Ch-4009 Basel, Switzerland.
- SELWITZ, R. H., ISMAIL, A. I. & PITTS, N. B. 2007. Dental caries. *Lancet*, 369, 51-59.
- SHAPIRO, S. & GUGGENHEIM, B. 1995. The action of thymol on oral bacteria. *Oral microbiology and immunology*, 10, 241-246.
- SINGH, K. A. & SPENCER, A. J. 2004. Relative effects of pre- and post-eruption water fluoride on caries experience by surface type of permanent first molars. *Community Dentistry and Oral Epidemiology*, 32, 435-446.
- SKENE, L. 2002. Ownership of human tissue and the law. *Nature Reviews Genetics*, 3, 145-148.
- STOOKEY, G. K., FEATHERSTONE, J. D. B., RAPOZO-HILO, M., SCHEMEHORN, B. R., WILLIAMS, R. A., BAKER, R. A., BARKER, M. L., KAMINSKI, M. A., MCQUEEN, C. M., AMBURGEY, J. S., CASEY, K. & FALLER, R. V. 2011. The Featherstone laboratory pH cycling model: A prospective, multi-site validation exercise. *American Journal of Dentistry*, 24, 322-328.
- SWAIN, M. V. & XUE, J. 2009. State of the Art of Micro-CT Applications in Dental Research. *International Journal of Oral Science*, 1, 177-188.
- TEN BOSCH, J. J. & ANGMAR-MANSSON, B. 1991. A Review of Quantitative Methods for Studies of Mineral Content of Intra-oral Incipient Caries Lesions. *Journal of Dental Research*, 70, 2-14.
- TEN CATE, J. M. 1990. In vitro Studies on the Effects of Fluoride on De- and Remineralization. *Journal of Dental Research*, 69, 614-619.
- TEN CATE, J. M., BUIJS, M. J. & DAMEN, J. J. M. 1995. pH-cycling of enamel and dentin lesions in the presence of low concentrations of fluoride. *European Journal of Oral Sciences*, 103, 362-367.
- TEN CATE, J. M., EXTERKATE, R. A. M. & BUIJS, M. J. 2006. The relative efficacy of fluoride toothpastes assessed with pH cycling. *Caries Research*, 40, 136-141.
- TEN CATE, J. M., LARSEN, M. J., PEARCE, E. I. F., FEJERSKOV, O., KIDD, E. A. M. & 2008. *Dental Caries the Disease and Its Clinical Management.* Oxford: Blackwell Munksgaard.

- TERUEL, J. D., ALCOLEA, A., HERNANDEZ, A. & RUIZ, A. J. O. 2015. Comparison of chemical composition of enamel and dentine in human, bovine, porcine and ovine teeth. *Archives of Oral Biology*, 60, 768-775.
- TOUMBA, K. J., AL-IBRAHIM, N. S. & CURZON, M. E. J. 2009. A review of slow-release fluoride devices. *European archives of paediatric dentistry : official journal of the European Academy of Paediatric Dentistry*, 10, 175-82.
- TUBERT-JEANNIN, S., AUCLAIR, C., AMSALLEM, E., TRAMINI, P., GERBAUD, L., RUFFIEUX, C., SCHULTE, A. G., KOCH, M. J., REGEWALTHER, M. & ISMAIL, A. 2011. Fluoride supplements (tablets, drops, lozenges or chewing gums) for preventing dental caries in children. *Cochrane Database of Systematic Reviews*.
- TWETMAN, S. 2009. Caries prevention with fluoride toothpaste in children: an update. *European archives of paediatric dentistry : official journal of the European Academy of Paediatric Dentistry*, 10, 162-7.
- TWETMAN, S., AXELSSON, S., DAHLGREN, H., HOLM, A. K., KALLESTAL, C., LAGERLOF, F., LINGSTROM, P., MEJARE, I., NORDENRAM, G., NORLUND, A., PETERSSON, L. G. & SODER, B. 2003. Caries-preventive effect of fluoride toothpaste: a systematic review. *Acta Odontologica Scandinavica*, 61, 347-355.
- TWETMAN, S., PETERSSON, L. G., AXELSSON, S., DAHLGREN, H., HOLM, A. K., KALLESTAL, C., LAGERLOF, F., LINGSTROM, P., MEJARE, I., NORDENRAM, G., NORLUND, A. & SODER, B. 2004. Caries-preventive effect of sodium fluoride mouthrinses: a systematic review of controlled clinical trials. *Acta Odontologica Scandinavica*, 62, 223-230.
- VIEIRA, A. E. D., DELBEM, A. C. B., SASSAKI, K. T., RODRIGUES, E., CURY, J. A. & CUNHA, R. F. 2005. Fluoride Dose Response in pH-Cycling Models Using Bovine Enamel. *Caries Research*, 39, 514-520.
- VOGEL, G. L. 2011. Oral fluoride reservoirs and the prevention of dental caries. *Monographs in Oral Science*, 22, 146-157.
- WHITE, D. J. 1992. THE COMPARATIVE SENSITIVITY OF INTRAORAL, INVITRO, AND ANIMAL-MODELS IN THE PROFILE EVALUATION OF TOPICAL FLUORIDES. *Journal of Dental Research*, 71, 884-894.
- WHITE, D. J. 1995. The application of in vitro models to research on demineralization and remineralization of the teeth. *Advances in dental research*, 9, 175-93; discussion 194-7.
- WHITE, D. J., FALLER, R. V. & BOWMAN, W. D. 1992. Demineralization and remineralization evaluation techniques - added considerations. *Journal of Dental Research*, 71, 929-933.
- WHITFORD, G. M. 2011. Acute Toxicity of Ingested Fluoride. In: BUZALAF, M. A. R. (ed.) *Fluoride and the Oral Environment*. Basel: Karger.
- WHO 2010. WHO Guidelines Approved by the Guidelines Review Committee. *Inadequate or excess fluoride: a major public health concern*. Geneva: World Health Organization.
- WHO 2015. Guideline: Sugars intake for adults and children. Geneva: World Health Organization.
- WILLERSHAUSEN, I., SCHULTE, D., AZARIPOUR, A., WEYER, V., BRISENO, B. & WILLERSHAUSEN, B. 2015. Penetration Potential of a Silver Diamine Fluoride Solution on Dentin Surfaces. An Ex Vivo Study. *Clinical Laboratory*, 61, 1695-1701.
- YAMAGA, R., NISHINO, M., YOSHIDA, S. & YOKOMIZO, I. 1972. Diamine silver fluoride and its clinical application. *The Journal of Osaka University Dental School*, 12, 1-20.

- YASSEN, G. H., PLATT, J. A. & HARA, A. T. 2011. Bovine teeth as substitute for human teeth in dental research: a review of literature. *Journal of oral science*, 53, 273-82.
- YEE, R., HOLMGREN, C., MULDER, J., LAMA, D., WALKER, D. & VAN PALENSTEIN HELDERMAN, W. 2009. Efficacy of silver diamine fluoride for Arresting Caries Treatment. *J Dent Res*, 88, 644-7.
- YEUNG, C. A., CHONG, L. Y. & GLENNY, A. M. 2015. Fluoridated milk for preventing dental caries. *Cochrane Database of Systematic Reviews*, 31.
- YOURAVONG, N., CARLEN, A., TEANPAISAN, R. & DAHLEN, G. 2011. Metal-ion susceptibility of oral bacterial species. *Letters in Applied Microbiology*, 53, 324-328.
- YU, D. G., KIMURA, Y., FUJITA, A., HOSSAIN, M., KINOSHITA, J. I., SUZUKI, N. & MATSUMOTO, K. 2001. Study on acid resistance human dental enamel and dentin irradiated by semiconductor laser with Ag(NH₃)₂F solution. *J Clin Laser Med Surg*, 19, 141-146.
- YU, O. Y., ZHAO, I. S., MEI, M. L., LO, E. C. M. & CHU, C. H. 2018. Caries-arresting effects of silver diamine fluoride and sodium fluoride on dentine caries lesions. *Journal of Dentistry*, 78, 65-71.
- ZERO, D. T. 1995. In situ caries models. *Advances in Dental Research*, 9, 214-230.
- ZERO, D. T., RAHBEK, I., FU, J., PROSKIN, H. M. & FEATHERSTONE, J. D. B. 1990. Comparison of the iodide permeability test, the surface microhardness test, and mineral dissolution of bovine enamel following acid challenge. *Caries Research*, 24, 181-188.
- ZHAO, I. S., GAO, S. S., HIRAISHI, N., BURROW, M. F., DUANGTHIP, D., MEI, M. L., LO, E. C. M. & CHU, C. H. 2018. Mechanisms of silver diamine fluoride on arresting caries: a literature review. *International Dental Journal*, 68, 67-76.
- ZHAO, I. S., MEI, M. L., LI, Q. L., LO, E. C. M. & CHU, C. H. 2017. Arresting simulated dentine caries with adjunctive application of silver nitrate solution and sodium fluoride varnish: an invitro study. *International Dental Journal*, 67, 206-214.
- ZHI, Q. H., LO, E. C. & KWOK, A. C. 2013. An in vitro study of silver and fluoride ions on remineralization of demineralized enamel and dentine. *Australian Dental Journal*, 58, 50-6.

Appendix

Appendix 1- Optimisation of dentine artificial subsurface lesion creation.

Days of de-mineralisation	Level of indentation from lesion surface (μm)												
	20	40	60	80	100	120	140	160	180	200	220	240	260
3	20.20	26.25	38.00	37.25	53.70	56.65	57.35	52.80	59.90	55.10	58.35	57.05	68.65
S.D	0.99	5.73	0.71	3.75	6.79	3.18	4.03	2.40	2.40	1.70	4.60	8.41	5.87
4	13.10	19.17	32.43	35.93	41.33	46.53	46.93	53.93	54.73	59.23	58.03	49.73	51.63
S.D	1.93	3.68	3.22	6.69	4.24	2.32	12.75	5.28	5.35	12.18	8.89	4.20	7.07
5	12.97	20.20	19.87	22.47	32.13	39.23	35.07	48.03	47.63	55.40	51.73	52.07	52.07
S.D	4.21	6.95	4.97	1.93	4.44	6.92	3.90	0.95	2.74	9.69	10.46	6.29	4.86
6	10.53	17.63	28.80	34.53	46.00	52.03	50.40	51.10	49.27	50.80	57.27	61.60	59.43
S.D	0.15	3.27	1.05	9.74	11.96	6.30	3.72	5.63	8.38	1.91	4.82	6.22	8.30
7	7.30	8.23	12.87	19.27	21.30	26.30	27.33	34.83	36.90	47.67	55.53	56.87	67.00
S.D	1.73	1.50	2.81	2.00	1.08	3.66	8.08	2.48	2.46	9.29	10.95	6.21	10.93