

**The Efficacy of Modern Remineralising Therapies Containing  
Calcium Phosphate in Remineralising Enamel Subsurface  
Lesions.**

**Shaikhah M A M Alotaibi**

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

**The University of Leeds**

**Leeds Dental Institute**

**Division of Child Dental Health**

**2018**

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

**This copy has been supplied on the understanding that it is copyright material and that no quotation from the thesis may be published without proper acknowledgement.**

## **Dedication**

**To my supportive parents**

**To my loving husband, Hussain**

**To my lovely children (Hassan and Maryam)**

**I love you more than anything and I appreciate all your patience and  
support**

## ACKNOWLEDGEMENTS

I am grateful to everyone who has helped me in my struggle to achieve this work. I shall begin with God the almighty: without his will and mercy I would have never accomplished this work. Then I would like to express my appreciation to Dr Jinous Tahmassebi for her persistent advice, encouragement and supervision, without her support and belief in my capability this work could not have been completed. You probably have no idea how blessed I felt to have you as my teacher. Thank you for your patience and effort.

I would also like to thank Professor Monty Duggal, my second supervisor, for his advice, comments, and the opportunity to work with him and to experience the most valuable and challenging learning process during these years.

I would also like to thank Dr Marina Malinowski, Dr Simon Strafford, and Sue Keat for their kind assistance with the laboratory procedures and Dr Jianhua Wu for the statistical advice.

A special thanks to my classmates, Amal Albalooshy, Marwah Albahrani, Jarrah Alkandari, Ibrahim Alshekaili, Abdulwadood Sharqawi, Tasneem Alwuhaib, and Evangelia Xiarchou for the numerous hours spent together sharing thoughts, concerns, and laughter. The best of luck to you in all of your endeavours.

I would like to express my gratitude to my family for their love and blessing. To my parents, your unconditional love, support, and encouragement has meant the world to me. I hope that I have made you proud.

To my best friend, my soulmate and my husband, Hussain, who was always helpful and motivating me to work toward the better at each step of my life, "Thank you" I simply could not have done this without you. I love you for everything, for being so understanding and supportive. I thank God for enlightening my life with your presence, you are the best thing that has ever happened to me.

To my two lovely children Hassan and Maryam, the pride and joy of my life, you have made me stronger, better, and more fulfilled than I could have ever imagined. I love you to the moon and back.

## ABSTRACT

### **Aims:**

To investigate *in vitro* the effect of Calcium Phosphate delivering technologies: Tooth Mousse (10% w/v CPP-ACP), MI paste Plus (10% w/v CPP-ACP, 900 ppm F), Clinpro 5000 (Tri-Calcium Phosphate, 5000 ppm F), Clinpro Tooth Crème ( Tri-Calcium Phosphate, 950 ppm F) in the remineralisation of artificial enamel subsurface lesions of bovine teeth and compare it with the effect of 1450 ppm F, 5000 ppm F, and 0 ppm F toothpastes using Quantitative Light-induced Fluorescence (QLF) technology.

### **Materials and Methods:**

Artificial enamel subsurface lesions were created in bovine enamel slabs (N=182) which were assigned randomly to seven groups; (1) Fluoride-free Toothpaste (negative control), (2) 1450 ppm F toothpaste (positive control), (3) 5000 ppm F toothpaste, (4) 1450 ppm F toothpaste + Tooth Mousse (10% w/v CPP-ACP), (5) 1450 ppm F toothpaste + MI Paste Plus (10% w/v CPP-ACP, 900 ppm F), (6) Clinpro 5000 (Tri-Calcium Phosphate, 5000 ppm F), and (7) Clinpro Tooth Crème ( Tri-Calcium Phosphate, 950 ppm F). All the experimental products were used according to the manufacturer recommendation of dosage. The enamel slabs were cycled for 28 days, QLF images were taken at the baseline and at the endpoint of the experiment. Data analysis was carried out using one-way analysis of variance (One way ANOVA).

**Results:** There were significant improvements in QLF values with all remineralising agents at the end of the study compared with baseline values within each group ( $P < 0.05$ ). All groups showed a statistically significant improvement in  $\Delta Q$  and Area of the lesion values compared with negative control (0 ppm fluoride) ( $P < 0.05$ ). In term of  $\Delta F$  values measurement, Clinpro Tooth crème was significantly more effective than high fluoride toothpaste (5000 ppm F) ( $P < 0.05$ ). 5000 ppm fluoride toothpaste was not different significantly to either Clinpro 5000 or the fluoride-free toothpaste ( $P > 0.05$ ).

**Conclusion:**

All group products were significantly more effective as remineralising agents of artificial early enamel subsurface lesions as compared with the negative control 0 ppm F. Calcium Phosphate delivering technologies; CPP-ACP with and without fluoride and Tri-Calcium Phosphate products were as effective as fluoridated toothpastes (1450 ppm F and 5000 ppm F) in remineralising artificial early enamel subsurface lesions. Tooth Mousse was as effective as MI Paste Plus when used in conjunction with 1450 ppm F toothpaste in remineralising artificial early enamel subsurface lesions in bovine teeth assessed using QLF technology.

## Table of Contents

<b>ACKNOWLEDGEMENTS .....</b>	<b>4</b>
<b>ABSTRACT .....</b>	<b>6</b>
<b>LIST OF TABLES .....</b>	<b>12</b>
<b>LIST OF FIGURES .....</b>	<b>13</b>
<b>INTRODUCTION .....</b>	<b>15</b>
<b>REVIEW OF LITERATURE .....</b>	<b>17</b>
1.1. Dental Caries.....	17
1.1.1. Demineralisation and Remineralisation.....	18
1.1.2. Enamel Caries.....	18
1.1.3. Role of Saliva.....	19
1.2. Enamel Remineralisation Therapies.....	20
1.2.1. Fluoride.....	20
1.2.2. Milk Products and enamel remineralisation .....	25
1.2.3. Calcium Phosphate Technologies .....	25
1.3. Model Systems Used to Study Caries Process in Enamel .....	37
1.3.1. <i>In Vitro</i> Models.....	37
1.3.2. Animal models.....	38
1.3.3. <i>In Situ Models</i> .....	39
1.4. Methods Used for Detecting Caries Lesion.....	40
1.4.1. Caries detection based on visual examination .....	40
1.4.2. X-Ray based Methods.....	41
1.4.3. Light-Emitting Devices.....	42



1.5. Research Aims and Hypotheses.....	46
1.5.1. Aims.....	46
1.5.2. The null hypotheses.....	47
<b>MATERIALS AND METHODS .....</b>	<b>48</b>
2.1. Power Calculation.....	48
2.2. Preparation of enamel slabs.....	48
2.3. Preparation of Enamel Subsurface lesions.....	52
2.4. Quantitative Light-induced Fluorescence imagery.....	53
2.5. $\Delta F$ range of the artificial lesions.....	57
2.6. Experimental and control groups.....	58
2.7. Experimental Materials.....	59
2.8. Toothpaste slurry.....	63
2.9. Randomisation and Blinding.....	63
2.10. The pH Cycling Regime.....	64
2.11. Flow Charts.....	66
2.12. Preparation of solutions.....	68
2.12.1. Artificial Saliva.....	68
2.12.2. Acetic Acid Preparation.....	71
2.13. Training and calibration.....	71
2.14. Intra-Examiner Reliability.....	72
2.15. Statistical Analysis of the Data.....	72
<b>RESULTS .....</b>	<b>73</b>
3.1. The mean fluorescence loss $\Delta F$ .....	73
3.1.1. Difference in $\Delta F$ within each group.....	74
3.1.2. Difference in $\Delta F$ between all groups.....	76

3.1.3. The percentage reduction in $\Delta F$ .....	80
3.1.4. Intra-examiner reproducibility for $\Delta F$ .....	80
3.2. The mean $\Delta Q$ : Lesion volume.....	81
3.2.1. Difference in $\Delta Q$ within each group.....	81
3.2.2. Difference in $\Delta Q$ between groups.....	84
3.2.3. The percentage changes in $\Delta Q$ .....	88
3.2.4. Intra-examiner reproducibility for $\Delta Q$ .....	88
3.3. Area of the white spot lesions .....	89
3.3.1. Difference in lesion area within each group .....	89
3.3.2. Difference in lesion area between groups.....	92
3.3.3. The percentage change in lesion area .....	95
3.3.4. Intra-examiner reproducibility for area of the white spot lesion .....	96
3.4. Summary of the results for all three parameters .....	97
<b>DISCUSSION.....</b>	<b>99</b>
4.1. <i>In vitro</i> models .....	99
4.2. Study design.....	100
4.3. Bovine teeth .....	100
4.4. Enamel slabs preparation and storage.....	101
4.5. Artificial caries lesions.....	102
4.6. pH cycling .....	103
4.7. Quantitative Light-induced Fluorescence .....	104
4.8. Results of Quantitative Light-induced Fluorescence.....	107
4.9. Remineralising potential of fluoridated toothpastes on early enamel subsurface lesions .....	107
4.10. Remineralising potential of Calcium Phosphate products on early subsurface lesions.....	111

4.11. Suggestion of future research .....	117
4.12. Null hypotheses .....	118
<b>CONCLUSION</b> .....	<b>119</b>
<b>REFERENCES</b> .....	<b>120</b>
<b>APPENDICES</b> .....	<b>XIV</b>

## LIST OF TABLES

Table 1: Composition of day time artificial saliva .....	69
Table 2: Composition of night time artificial saliva .....	70
Table 3: Composition of acetic acid solution .....	71
Table 4: Paired sampled T-Test results for values of $\Delta F$ -Baseline and $\Delta F$ -Endpoint .....	75
Table 5: Multiple comparisons of $\Delta F$ -Differences between groups with Bonferroni correction .....	79
Table 6: Paired sampled T-Test results for $\Delta Q$ values at baseline and after treatment .....	82
Table 7: One-way ANOVA between groups for the $\Delta Q$ -Difference .....	86
Table 8: Multiple comparisons of $\Delta Q$ -Difference between test groups ....	87
Table 9: One-way ANOVA between groups for the values of Area-Baseline for all groups .....	89
Table 10: Paired sampled T test results for the lesion area values at baseline and after treatment for all groups .....	90
Table 11: Multiple comparisons of Area-Differences between all groups with Bonferroni correction .....	94

## LIST OF FIGURES

<b>Figure 1: Diamond wire saw apparatus used for teeth sectioning (Well® Walter EBNER, CH-2400 Le Loche) .....</b>	<b>49</b>
<b>Figure 2: Enamel slab attached to the plastic spoon with sticky wax and suspended in the plastic universal tube .....</b>	<b>50</b>
<b>Figure 3: Enamel slab covered with nail varnish except for a small window.....</b>	<b>51</b>
<b>Figure 4: Enamel slabs stored in plastic universal tubes .....</b>	<b>52</b>
<b>Figure 5: Enamel Slab immersed in acidified hydroxyethyl cellulose gel for 10 days to create a subsurface caries-like lesion .....</b>	<b>53</b>
<b>Figure 6: QLF machine and camera fixed in position on a stand.....</b>	<b>55</b>
<b>Figure 7: QLF image taken with the blue light showing a patch drawn in sound enamel around the lesion with the demineralised lesion in the centre of the enamel slab.....</b>	<b>56</b>
<b>Figure 8: Fluoride-free toothpaste (0 ppm fluoride)- Boots Smile Non Fluoride.....</b>	<b>60</b>
<b>Figure 9: Sodium Fluoride toothpaste 1450 ppm – Colgate Cool Stripe cavity protection .....</b>	<b>60</b>
<b>Figure 10: Duraphat Toothpaste 5000 ppm Fluoride as Sodium Fluoride – Colgate Duraphat 1.1% W/W .....</b>	<b>61</b>
<b>Figure 11: GC Tooth Mousse – Topical crème with bio-available calcium and phosphate – 10% w/v CPP-ACP.....</b>	<b>61</b>

<b>Figure 12: GC MI Paste Plus with 900 ppm Fluoride – Topical crème with calcium phosphate and fluoride – 10% CPP-ACP &amp; 0.2% w/w sodium fluoride).....</b>	<b>62</b>
<b>Figure 13: Clinpro 5000 – Tri-Calcium Phosphate with 5000 ppm Fluoride – 1.1% Sodium Fluoride (3M ESPE dental, United Kingdom).....</b>	<b>62</b>
<b>Figure 14: Clinpro Tooth Crème – Tri-Calcium Phosphate with 950 ppm Fluoride – 0.21% Sodium Fluoride (3M ESPE dental, United Kingdom) .....</b>	<b>63</b>
<b>Figure 15: Flow chart for 0 ppm F, 1450 ppm F, 5000 ppm, Clinpro Tooth Crème, and Clinpro 5000 toothpastes.....</b>	<b>66</b>
<b>Figure 16: Flow chart for 1450 ppm F + Tooth Mousse and 1450 ppm F + MI Paste Plus .....</b>	<b>67</b>
<b>Figure 17: Mean values of <math>\Delta F</math>- Baseline and <math>\Delta F</math>-Endpoint for all groups .</b>	<b>76</b>
<b>Figure 18: Means of the <math>\Delta F</math>-Difference of all groups .....</b>	<b>77</b>
<b>Figure 19: The % reduction in <math>\Delta F</math> values for all groups .....</b>	<b>80</b>
<b>Figure 20: The means of both <math>\Delta Q</math>-Baseline and <math>\Delta Q</math>-Endpoint values for all groups.....</b>	<b>83</b>
<b>Figure 21: Means of the <math>\Delta Q</math>-Difference of all groups .....</b>	<b>85</b>
<b>Figure 22: The % change in <math>\Delta Q</math> for all groups .....</b>	<b>88</b>
<b>Figure 23: Lesion Area at baseline and after treatment for all groups.....</b>	<b>91</b>
<b>Figure 24: Means of the lesion Area-Difference of all tested groups .....</b>	<b>92</b>
<b>Figure 25: The % reduction in Area values for all groups .....</b>	<b>95</b>

## INTRODUCTION

In most high income countries, dental caries is considered as a major public health problem, and it affects about 60–90% of school children (Petersen and Lennon, 2004). Over the last 30 years, the prevalence of dental caries has remarkably decreased worldwide. This decline was mainly attributed to the use of oral fluoride products. Fluoride is considered as a fundamental contributor to remineralisation and the gold standard against which other agents have to compete (Zero, 2006). Fluoride's presence during remineralisation promotes the formation of fluorapatite in enamel. Research has shown that one unit cell of fluorapatite requires two fluoride ions, ten calcium ions and six phosphate ions (Reynolds, 2008). Therefore, the amount of calcium and phosphate ions available in saliva during the process of remineralisation determines the net enamel remineralisation (Cochrane et al., 2010). Consequently, dental products containing calcium, phosphate, and fluoride were proposed and were claimed to be more effective over fluoride only products as it provides an external source of calcium and phosphate that is needed for remineralisation (Shen et al., 2011). However, the combination of these three ions; calcium, phosphate and fluoride in one product could lead to an unfavourable reaction between them rendering the product ineffective (Sullivan et al., 2001). Recently two technologies were introduced and claimed to overcome this incompatibility. The first technology is Casein Phosphopeptide Amorphous Calcium Phosphate (CPP-ACP) delivered either as 'Tooth Mousse' or 'MI Paste Plus' which contains 900 ppm F (Cochrane et al., 2010). The second technology is Tri-Calcium Phosphate which has been exclusively introduced by

3M ESPE. Currently, it is incorporated into many products such as Clinpro 5000 toothpaste with 5000 ppm F, Clinpro Tooth Crème with 950 ppm F, and Clinpro White Varnish with 22600 ppm F (Karlinsky et al., 2010). In the literature evidence is sparse for the effectiveness of fluoride only products compared with calcium phosphates combined with fluoride. Therefore, the aim of this study was to determine the effectiveness of Calcium Phosphate technologies in Tooth Mousse, MI Paste Plus, Clinpro 5000, and Clinpro Tooth Crème in comparison with fluoride products; 1450 ppm F, and 5000 ppm F or 0 ppm F toothpastes in remineralising artificial enamel subsurface lesions in bovine teeth *in vitro* using Quantitative Light-induced Fluorescence technology.



## Chapter 1

### REVIEW OF LITERATURE

#### 1.1. Dental Caries

Dental caries is a chronic, infectious, multifactorial disease of the teeth calcified tissues characterised by demineralisation of the inorganic portion by acidic by-products from bacterial fermentation of carbohydrates followed by destruction of organic substance leading to cavitation. For the dental caries to progress, an interaction between pathological factors represented by dental plaque biofilm and cariogenic diet with protective factors such as saliva over time should take place first. Dental plaque contains acidogenic bacteria that utilise fermentable carbohydrate and produce acid as a by-product of metabolism. These are mainly *Streptococcus Mutans* and *Lactobacilli* and are considered the primary causative agents of dental caries (Featherstone, 2000).

The second pathological factor is the cariogenic diet containing fermentable carbohydrates such as glucose, sucrose, fructose or cooked starch. It is well known that ingestion of these fermentable carbohydrates is the most significant contributing factor in the development of dental caries and a strong correlation between the two has been shown (Hankin et al., 1973). In dental plaque, once pH drops below the critical pH (about 5.5) by the interaction of the pathological factors, demineralisation of dental hard tissue takes place (Featherstone, 2000). Then protective factors take over and the

pH is restored back to normal. These protective factors are saliva, fluoride, tooth brushing, and reducing the frequency of ingestion of cariogenic diet (Featherstone, 2004).

### **1.1.1. Demineralisation and Remineralisation**

Demineralisation and remineralisation are two dynamic processes that simultaneously occur in the oral cavity. The demineralisation process is governed by microbiological shifts within the dental biofilm, and is initiated when acids produced by endogenous bacteria diffuse into hard tooth structure and dissolve the hydroxyapatite crystals. Remineralisation, on the other hand, occurs when minerals and the pH of the biofilm are restored by salivary buffers (Featherstone, 2000).

The dynamic balance between the two processes determines the degree and rate of caries progression or regression. If the balance between remineralisation and demineralisation is shifted to the demineralisation then the caries process begins. This caries process in its early stage can be arrested or even reversed if the imbalance between remineralisation and demineralisation is restored (Featherstone, 2000).

### **1.1.2. Enamel Caries**

Enamel is the hardest calcified tissue in the body and represents the outermost covering of the tooth structure. It is an acellular tissue that consists by volume of 85% inorganic hydroxyapatite, 12% water, and 3% organic material; proteins and lipids (Robinson et al., 2000). The enamel structure is composed of millions of long prisms and rods extending from the enamel-dentine junction to the outer enamel surface. This high mineral content

enables the tooth to withstand the high masticatory force and this is the main function of enamel. Despite this rigidity, enamel acts as a permeable surface as it permits the passage of small molecules and different minerals such as remineralising agents (Featherstone, 1999).

Early enamel caries presents clinically with an intact surface as a white opacity known as a white-spot lesion (Silverstone, 1973).

Darling (1961; 1956) described four distinct histopathologic zones of the early enamel lesion:

- 1- Translucent zone: represents the deepest part of the lesion where the first visible carious change in the enamel occurs due to the loss of at least 1-2% mineral. Protein removal occurs first followed by loss of inorganic ions.
- 2- Dark zone: situated superficial to translucent zone where porosity increases about 5-10%.
- 3- Body of the lesion: situated beneath the intact enamel surface and represents the majority of the lesion in which further demineralisation results in about 25-50% porosity.
- 4- Surface zone: represents the intact translucent surface which covers the lesion. Porosity of this surface zone is about 1-2% which is similar to the porosity of intact enamel.

### **1.1.3. Role of Saliva**

One of the critical protective factors that favours remineralisation of dental hard tissue in the oral cavity is saliva. Saliva has a pH of around 6.8-7 in healthy patients thus capable of neutralising and buffering the bacterial acid (Azrak et al., 2003). There are three buffer systems in saliva; the protein

buffer, phosphate buffer, and the bicarbonate buffer. The latest is the major buffer in stimulated saliva. Once food is consumed and pH drops, a rise in bicarbonate concentration occurs. This increased level of bicarbonate in stimulated saliva during pH drop diffuses into the dental plaque and helps neutralise the acid by binding to H<sup>+</sup> (Lenander-Lumikari and Loimaranta, 2000). Saliva also slows down the caries process by acting as a reservoir of calcium and phosphate ions that can replace those dissolved from the tooth during acid attack. Another important function of saliva is to clean and wash the oral cavity from different substances introduced into the mouth. Saliva also act as a lubricant and has anti-microbial properties (Stookey, 2008). It contains several potentially important antibacterial agents such as immunoglobulin, lactoferrin, and lyzosome which interfere with bacterial adhesion and enhance clearance of bacteria from the mouth (Moslemi et al., 2015).

## **1.2. Enamel Remineralisation Therapies**

Reversing the process of demineralisation has been widely investigated and this has led to the development of many effective remineralising technologies such as fluoride products and calcium phosphate products (Jindal et al., 2015).

### **1.2.1. Fluoride**

The caries-inhibiting effect of fluoride has been known since the 1930s when the difference in caries prevalence between communities with and without naturally occurring fluoride in drinking water was evident. After the

introduction of fluoridated toothpastes, a dramatic decrease in worldwide caries level has been noticed (Featherstone, 1990). The effect of fluoride in promoting remineralisation and preventing caries is well established (Lynch et al., 2004, Bowen, 1995).

#### **1.2.1.1. Fluoride Mechanism of Action**

Fluoride is believed to influence the oral cavity in a number of ways. First of all, during tooth development, the presence of fluoride ions enhances the formation of fluorapatite instead of hydroxyapatite. It is well documented that fluorapatite is more stable and more resistant to dissolution by acid than hydroxyapatite (Featherstone, 2000).

After tooth eruption, teeth can be frequently exposed to acid attack every time fermentable carbohydrate is metabolized by oral bacteria. As a result pH drops leading to demineralisation of the tooth hard tissue. However, if fluoride is present in the oral fluid at the time the bacteria produce acid it will diffuse along with the acid to the tooth sub-surface and inhibit demineralisation (Featherstone, 1990).

Fluoride also enhances remineralisation by attracting both calcium and phosphate into the early enamel subsurface lesions promoting the growth of a new surface.

Moreover, fluoride ion has a bactericidal property and in low pH it binds to hydrogen ions forming hydrogen fluoride which diffuses into the bacteria inhibiting the essential enzyme enolase (Hamilton, 1990).

### **1.2.1.2. Fluoride delivery systems**

Different vehicles were developed to deliver fluoride and they can be divided into systemic or topical technologies. Water is the first discovered natural vehicle for delivering fluoride systemically (WHO, 2006). Water fluoridation was found to reduce caries up to 70% (Burt and Eklund, 1999). According to the World Health Organisation, water fluoridation is considered as the fundamental part of the basic human rights (Petersen and Lennon, 2004). Other systemic fluoride delivering technologies are tablets and drops. They are recommended by the American Academy of Paediatric Dentistry (AAPD) for children who drink fluoride-deficient water (AAPD, 2015). Their use in children has been strongly linked to caries reduction (Tubert-Jennin et al., 2011).

Other vehicles have also been developed through which fluoride can be delivered topically. An example is fluoride varnish which is safe, very effective, and delivers concentrated amounts of fluoride (Marya and Dahiya 2006). Marinho et al. (2013), in their Cochrane review reported that fluoride varnish achieved 43% reduction in DMFS and 37% reduction in dmft.

Furthermore, gels and rinses have been used to deliver fluoride and about 40% reduction in caries by fluoride mouth rinse and 21% reduction in DMFT by fluoride gel have been found according to a Cochrane review by Marinho et al. (2002).

Another very important fluoride technology is toothpaste which is the most commonly used fluoride delivery method worldwide. Since introduced, there has been remarkable reduction in the prevalence of dental caries. Twetman

et al. (2003), in his systematic review of 54 papers, showed a strong evidence of caries prevention highly linked to the daily use of fluoride toothpaste in the young permanent dentition.

A Cochrane review comparing fluoride toothpaste with placebo in children up to 16 years during at least 1 year provides clear evidence that fluoride toothpastes are efficacious in preventing dental caries in the young permanent dentition (Marinho et al., 2003). This finding is consistent with the result of a more recent review carried out by Twetman (2009).

Moreover, this fluoride tool effectiveness was found to be concentration-dependent (Marinho et al., 2003). For example, the comparison of toothpaste with 1250, 2500, and 5000 ppm fluoride in children for three years in a clinical trial showed that 5000 ppm F toothpaste was significantly superior than the other concentrations in reducing DMF scores (Cutress et al., 1992). The maximum concentration in an over-the-counter products in Europe is the 1500 ppm F. High fluoride toothpastes, 2800 ppm F and 5000 ppm F, can only be prescribed by a dental professional and are beneficial to high risk caries group (Davies, 2008).

#### **1.2.1.3. Fluoride Limitation**

Fluoride promotes enamel remineralisation majorly through the formation of fluorapatite. It has been found that one unit cell of fluorapatite requires two fluoride ions, ten calcium ions and six phosphate ions. Therefore the amount of calcium and phosphate in saliva during fluoride application guides the net enamel remineralisation. Subsequently, inadequacy of calcium and phosphate even in the presence of high levels of fluoride ions cease the enamel remineralisation rendering the fluoride ineffective (Reynolds, 2008).

#### **1.2.1.4. Fluoride Toxicity and Dental Fluorosis**

It is well established that prolonged use of fluoride at recommended levels does not cause any serious harm to the human body. However, if fluoride is ingested in high concentrations that exceeds safe limits, harmful effects can occur. The probable toxic dose is said to be 5 mg of F/Kg of body weight (Whitford et al., 1990). Fluoride toxicity can be classified as acute and chronic toxicity. Symptoms of acute toxicity which can occur after a single ingestion of large amount of fluoride include abdominal pain, vomiting, nausea, diarrhoea, excess salivation, cardiac failure, convulsions or even death. Chronic toxicity on the other hand is caused due to prolonged ingestion of smaller amounts of fluoride. Symptoms of chronic toxicity include dental and skeletal fluorosis. Dental fluorosis or teeth mottling is irreversible and only occurs when exposure to fluoride occurs during enamel development. According to the AAPD the recommended daily intake of fluoride should be between 0.05 – 0.07 mg of fluoride /Kg of body weight (Buzalaf, 2011).

According to the European Academy of Paediatric Dentistry (2009), a fluoride preventive programme should fit the patient's needs and risks. The use of fluoride, therefore, must be balanced between maximising the caries-preventive effect and minimising the risk of toxicity and dental fluorosis.



### **1.2.2. Milk Products and enamel remineralisation**

Dairy products such as milk and cheese have been always linked to good oral health and their anticariogenic properties have been supported well in the literature (Krobicka et al., 1987, Rosen et al., 1984, Reynolds and Johnson, 1981). Cow's milk was found to be capable of remineralising enamel subsurface lesions (Mor and Rodda, 1983, Mcdougall, 1977). Shaw et al. (1959) found that rats fed only on bovine milk for 14-24 weeks did not develop any dental caries.

The anticariogenic properties of milk are mainly attributed to the presence of high concentrations of calcium and phosphate (Krobicka et al., 1987, Rosen et al., 1984, Reynolds and Johnson, 1981) along with Casein, a multi-phosphorylated protein, that stabilises milk calcium and phosphate and accounts for approximately 80% of the total milk protein (Aimuti, 2004). Reynolds and Rio (1984) suggested that Casein exerts its anticariogenic effect topically through binding to hydroxyapatite and preventing adherence of oral bacteria to enamel. Casein also is capable of preventing the dissolution of hydroxyapatite from enamel by increasing the acid-buffering capacity of dental plaque (Galhotra et al., 2014, Hipp et al., 1952).

### **1.2.3. Calcium Phosphate Technologies**

Calcium and Phosphate ions are essential for dental hard tissue strength. They play an important role in reducing the solubility of enamel and promoting teeth remineralisation (Li et al., 2014). The literature showed that the level of calcium and phosphate is inversely associated with caries

incidence which indicates a protective effect of salivary calcium and phosphate against dental caries (Sewon and Makela, 1990).

Moreover, fluoride's ability to promote remineralisation in the oral cavity is limited by the availability of calcium in the saliva (Cochrane, et al. 2010).

Therefore, in an approach to enhance the effectiveness of fluoride products, a number of calcium phosphate technologies were developed. They are claimed to be more effective in remineralising enamel lesions over fluoride products alone (Shen et al., 2011). These includes: Tooth Mousse, MI Paste Plus and Tri-Calcium Phosphate (tTCP).

#### **1.2.3.1. Casein Phosphopeptide-Amorphous Calcium Phosphate (CPP-ACP)**

CPP-ACP is a new calcium phosphate remineralisation technology which is derived from the milk protein Casein and acts as a reservoir of calcium and phosphate to promote enamel remineralisation (Roopa et al., 2015). It is the second most extensively studied technology after fluoride. CPP-ACP caries-preventive effect has been supported in many studies ranging from *in vitro*, *in situ* studies, and clinical trials (Walsh, 2009). CPP-ACP has been incorporated into dental cream which is commercially available as Tooth Mousse and MI Paste Plus (Jindal et al., 2015).

##### **1.2.3.1.1. *In Vitro* studies**

An *in vitro* study conducted by Zhang et al. (2011) to compare Tooth Mousse and 500 ppm Sodium Fluoride solution in remineralising artificial early enamel subsurface lesions of primary teeth found Tooth Mousse to be significantly more effective than 500 ppm Sodium Fluoride solution after 30

days of intervention. This effectiveness of Tooth Mousse as an enamel remineralising agent was confirmed by a more recent *in vitro* study conducted by Zhou et al. (2014) which showed that Tooth Mousse has a significantly more favourable remineralisation effect than Sodium Fluoride. It also found that the effect of Tooth Mousse was dependent on the length of application. The comparison between the remineralising potential of Tooth Mousse applied for 4, 6, 8, 12 and 24 hours on primary teeth showed that Tooth Mousse was effective in reducing enamel surface roughness. The maximum reduction of surface roughness was achieved by 24 hours application of Tooth Mousse. Crystal size was also assessed via x-ray diffraction which showed that the size of hydroxyapatite crystals increased after remineralisation with Tooth Mousse as compared to Sodium Fluoride. However, it is worthwhile to note that in the previous study the durations of Tooth Mousse application were long, and this is impractical and insensible in clinical practice as no patient will comply with it.

Moreover, a number of studies in the literature have shown that the combination of Tooth Mousse and fluoride achieved more remineralisation of enamel lesions. This synergic effect was evident in the rat caries model of Reynolds et al. (1995).

In an *in vitro* study, bovine teeth with artificial early enamel subsurface lesions were randomly divided into 4 groups as follows: Tooth Mousse, fluoridated toothpaste, fluoridated toothpaste followed by Tooth Mousse, and artificial saliva which acted as a control. At the end of the intervention period, which extended for 12 weeks, circularly polarized images were taken. The result demonstrated a decrease in the size of the early enamel subsurface lesions with time in all groups with the greatest reduction in sizes observed in

the group receiving fluoridated toothpaste followed by Tooth Mousse. It was concluded that Tooth Mousse can reduce the size of early enamel subsurface lesions and promote the remineralisation of bovine enamel and it appeared that the combined application with fluoride toothpaste strengthens the effect (Wu et al., 2010).

Elsayad et al. (2009) confirmed this synergy in his *in vitro* study. In his study, Tooth Mousse was compared with a combination of Tooth Mousse and 0.22% Fluoride. After 3 weeks of intervention, the artificial enamel lesions were assessed by Quantitative Light-Induced Fluorescence. Both interventions were effective as remineralising agents, and Tooth Mousse combined with fluoride was significantly more effective than without it. However, their finding was inconsistent with another *in vitro* study which found that Tooth Mousse was as effective as Fluoride Varnish in their remineralisation effect and the combination between Tooth Mousse and Fluoride failed to achieve any improvement in their effects (Lata et al., 2010).

#### **1.2.3.1.2. *In Situ* studies**

Shen et al. (2011) in his *in situ* trial showed Tooth Mousse and MI Paste Plus to be more effective than other fluoride products. Intra-oral appliances of human enamel specimens with early enamel subsurface lesions were worn by volunteers and exposed to one of the different test products for 60 seconds four times daily for 10 days. These products included 1000 ppm F, 5000 ppm F, Tooth Mousse, MI Paste Plus, and Clinpro Tooth crème with 950 ppm F. The final outcome showed that Tooth Mousse and MI Paste Plus significantly achieved more remineralisation of early enamel subsurface

lesions than 5000 ppm F toothpaste. It has been shown that Tooth Mousse achieved 24% remineralisation of enamel as compared with 29% by MI Paste Plus and only 16% by 5000 ppm F toothpaste. Moreover, salivary concentrations of calcium and phosphate were measured and they were significantly higher in Tooth Mousse and MI Paste Plus compared to Clinpro Tooth crème. In fact, the MI Paste Plus increased salivary calcium concentration 35 times higher than the Clinpro Tooth crème. Interestingly, a difference in the pattern of enamel remineralisation was observed. The study showed that fluoride tends to remineralise predominantly the superficial surface layer of enamel lesions. Tooth Mousse on the other hand is capable of incorporating minerals deeper into the subsurface where the body of the lesion is remineralised instead.

Additionally, a recent *in situ* study investigated the additional benefit of the use of Tooth Mousse over the use of fluoridated toothpaste. The subjects were randomly divided into 3 groups as follows: brushing with 1400 ppm F toothpaste followed by application of Tooth Mousse; brushing with 1400 ppm F toothpaste as positive control; brushing with 0 ppm F toothpaste as negative control. The test products were used twice daily for 4 weeks. The lesions were assessed by transverse microradiography. The final outcome showed that the 1400 ppm F toothpaste was significantly superior to the combination of 1400 ppm F toothpaste and Tooth Mousse. This combination was equivalent to 0 ppm F toothpaste. The additional use of Tooth Mousse appears to be less effective than the prolonged use of fluoridated toothpaste. In fact, Tooth Mousse was believed to hamper the remineralisation that was expected by 1400 ppm F toothpaste (Meyer-Lueckel et al., 2015). This could be due to the binding between fluoride from the fluoridated toothpaste and

calcium in the Tooth Mousse forming calcium fluoride which would be swallowed and not being available to exert any effects.

#### **1.2.3.1.3. *In Vivo* studies**

A randomised controlled trial conducted to investigate the effect of Tooth Mousse in the primary dentition found that Tooth Mousse twice a day for a year was as effective as fluoride varnish applied tri-annually. In this study, 140 children aged between 1- 3 years with white spot lesions in maxillary anterior teeth were selected and randomly divided into 4 groups. Group 1 received no treatment and acted as a control. In group 2, children received oral hygiene and dietary counselling. In group 3, in addition to oral hygiene, subjects received fluoride varnish 3 times a year at 4, 8, and 12 months. Group 4 subjects received oral hygiene and Tooth Mousse twice daily for a year. The size of white spot lesions as well as dmft indices were assessed at baseline, 4, 8, and 12 months post intervention and comparisons within and between groups were observed. The results showed that the prolonged use of Tooth Mousse significantly reduced white spot lesion size by 63% as compared to 51% by fluoride varnish (Memarpour et al., 2015).

Another randomised control trial conducted by Sim et al. (2015) aimed to determine the effect of Tooth Mousse on caries progression in patients with nasopharyngeal carcinoma demonstrated a caries-preventive effect of Tooth Mousse. In the study, patients were randomly divided into 2 groups. Both groups had 0.4% Stannous Fluoride gel once daily and dental crème three times daily for a 3 month period. Group 1 used a crème containing 10% CPP-ACP while group 2 used placebo crème without CPP-ACP. Caries status

was assessed before and after 3 months of intervention. The result demonstrated a lower rate of caries progression in the Tooth Mousse group compared with the other group. However, the difference was not statistically significant.

Tooth Mousse has also been used to treat patients with white spot lesions resulting from orthodontic treatment despite the conflicting results regarding its effectiveness over normal oral hygiene procedures. Bailey et al. (2009) for example showed in their randomised controlled trial that 10% CPP-ACP cream twice a day for 12 weeks was more effective than a placebo cream in reducing white spot lesions based on clinical assessments according to ICDAS II criteria. This was inconsistent with a more recent randomised controlled trial conducted by Brochner et al. (2011) which failed to demonstrate any additional effect of using Tooth Mousse over routine oral hygiene and tooth brushing with fluoridated toothpastes. In this study the participants were randomly divided into two groups. The subjects in the first group were instructed to brush their teeth with 1100 ppm F toothpaste in the morning and apply Tooth Mousse in the evening. The other group on the other hand were instructed to brush with 1100 ppm F toothpaste two times a day (morning and evening). The white spot lesion size was assessed at baseline and after 4 weeks by Quantitative Light-Induced Fluorescence. The result showed that 1100 ppm F toothpaste was as effective as Tooth Mousse in reducing the white spot lesions size after 4 weeks of intervention.

It should be kept in mind that in the study by Bailey and colleagues (2009), the participants received Tooth Mousse twice a day following tooth brushing with 1000 ppm F toothpaste, whereas in the study by Brochner et al (2011), the subjects were instructed to brush their teeth with 1100 ppm F toothpaste

in the morning and to apply Tooth Mousse only once in the evening without the use of F toothpaste. Consequently, the superiority of Tooth Mousse in the first study may very well be due to the influence of combining Tooth Mousse with fluoride, a calcium, phosphate, and fluoride could act synergistically to enhance more remineralisation effect on early enamel lesion (Elsayed et al., 2009). Furthermore, longer intervention period (12 weeks versus 4 weeks) as well as more application of Tooth Mousse (twice versus once daily) could have played a role too.

Li et al. (2014) carried out a systematic review, based on eight studies; it was found that CPP-ACP has a long-term remineralisation effect on early caries lesions in comparison with placebo, although this effect was not significantly different from that of fluoride agents. The review concluded that the clinical benefits of using CPP-ACP supplements over fluoride are still unclear; hence, well-designed RCTs are required to improve the level of evidence in this area.

#### **1.2.3.2. Casein Phosphopeptide- Amorphous Calcium Fluoride Phosphate**

CPP-ACFP was proposed based on the assumption that calcium and phosphate could synergistically work with fluoride to improve remineralisation of enamel lesions. This technology is available commercially as MI Paste Plus with 900 ppm F.

Reynolds et al., in 1995 conducted a study in which 144 animals were divided to 9 groups to compare between different concentration of Casein phosphopeptide and calcium phosphate (0.1%, 0.2%, 0.5%, and 1.0%) to 500 ppm sodium fluoride alone or when combined with 0.5% Casein



phosphopeptide with calcium phosphate. All solutions were applied to the animals' molar teeth twice daily. The result of the study demonstrated a dose-response effect. The 1.0% CPP was as effective in inhibiting caries formation as 500 ppm fluoride. The combination of fluoride, casein phosphopeptide, and calcium phosphate was more effective than CPP-ACP or fluoride alone and this supported the synergic effect of fluoride with CPP-ACP. The mechanism of caries prevention for CPP-ACP complexes was suggested to be through increasing the level of calcium phosphate in plaque, inhibiting enamel demineralisation and enhancing remineralisation.

Many studies in the literature have proven the superiority of CPP-ACFP over CPP-ACP in remineralising early enamel lesions. In a recent *in vitro* study conducted by Oliveira et al. (2014), human teeth were randomly assigned to four treatments for 30 days: control with no treatment, Tooth Mousse, 5000 ppm F toothpaste, and MI Paste Plus. Lesions were assessed at baseline, 10, 20, and 30 days post treatment with QLF to examine differences in both fluorescence loss and lesion area size. In terms of fluorescence loss, final comparison revealed no statistically significant differences between Tooth Mousse and MI Paste Plus at any of the follow-up time points. In terms of lesion area size observation, MI Paste Plus was more effective than Tooth Mousse indicating enhancement of remineralising potential by the addition of fluoride to Tooth Mousse.

Another recent *in vitro* study using DIAGNOdent™ to investigate the remineralisation achieved by Tooth Mousse and MI Paste Plus and compare it with artificial saliva found that both Tooth Mousse and MI Paste Plus showed statistically significant enamel remineralisation compared with the

artificial saliva. MI Paste Plus was more effective than Tooth Mousse (Jayarajan et al., 2011).

Shen et al. (2011) in his *in situ* trial showed MI Paste Plus to be more effective than Tooth Mousse. Intra-oral appliances of human enamel specimens with subsurface lesions were worn by volunteers and exposed to one of different test products for 60 seconds four times daily for 10 days. These products included 1000 ppm F, 5000 ppm F, Tooth Mousse, MI Paste Plus, and Clinpro Tooth Crème with 950 ppm F. Saliva samples were collected after rinsing with the remineralising agent. Calcium, phosphate, and fluoride levels in enamel were measured using ion chromatography and transverse microradiography. The final comparison revealed differences in salivary fluoride, calcium, and phosphate concentrations among the different remineralising agents. The highest salivary fluoride concentration was observed from the 5000 ppm F samples and this was about 5 times higher than that observed with 1000 ppm F, Clinpro, or MI Paste Plus. No significant difference was found between the last three products. Calcium and phosphate ion measurements also revealed significantly increased salivary calcium and phosphate concentrations related to both Tooth Mousse and MI Paste Plus compared to the other products. The following order of remineralisation from highest to lowest was demonstrated: MI Paste Plus, Tooth Mousse, 5000 ppm F, both 1000 ppm F and Clinpro Tooth Crème, then placebo.

Nevertheless, a conflicting result was found by an *in vitro* study by Mehta et al. (2013) who failed to demonstrate any difference in the remineralisation achieved by either MI Paste Plus or Tooth Mousse. Enamel lesions were

assessed using QLF at different time points. The intervention period extended to 21 days. The study showed that Tooth Mousse was as effective as MI Paste Plus in remineralising artificial enamel lesions throughout the study. Interestingly, it was found that after 14 days of remineralisation cycle, both MI Paste Plus and Tooth Mousse were equivalent to artificial saliva in their remineralisation effect on enamel lesions.

Beerens et al. (2010) in their randomised controlled trial which was conducted on orthodontic patients found no additional benefit of the use of MI Paste Plus on enamel white spot lesions when compared with normal oral hygiene procedures. The study extended for 12 weeks and the lesions were assessed by QLF after debonding and 6 and 12 weeks thereafter. A significant decrease in fluorescence loss was found with respect to baseline for both groups and no difference was found between groups. This confirmed the results of *in situ* study by Vanichvatana and Auychai (2013). The study could not find any additional benefit of MI Paste Plus compared with fluoridated toothpaste alone. Comparison between fluoridated toothpaste followed by application of MI Paste Plus with the sole use of fluoridated toothpaste showed that both were equivalent in their remineralisation effect.

#### **1.2.3.3. Tri-Calcium Phosphate**

Another calcium phosphate delivering technology is Tri-Calcium Phosphate. It is a new technology that delivers phosphate and calcium to the teeth which work synergistically with fluorides without any unfavourable interactions during storage of the product. It has been introduced by 3M ESPE and is incorporated into many products that are available in the market such as

Clinpro 5000 toothpaste with 5000 ppm F, Clinpro White Varnish with 22600 ppm F, and Clinpro Tooth Crème with 950 ppm F (Karlinsky et al., 2010).

The potential of Tri-Calcium Phosphate is promising; however its remineralising potential has been investigated by a limited number of studies. As yet no clinical trial has investigated its remineralisation effect.

The addition of calcium phosphate seems to improve the remineralisation effect of fluoride rather than fluoride alone. For example, an *in vitro* study by Karlinsky et al. (2010) showed that Clinpro 5000 was significantly more effective in remineralising both enamel surface and subsurface lesions than 5000 ppm F toothpaste. After 10 days the final comparison demonstrated that Clinpro 5000 produced 30% denser subsurface lesions as compared with 5000 ppm F toothpaste.

However, a more recent *in vitro* study conducted by Basappa et al. (2013) failed to demonstrate any additional effect of calcium phosphate over fluoride alone. The study compared between 5000 ppm F toothpaste, MI Paste Plus, and Clinpro Tooth Crème, and showed that 5000 ppm F toothpaste was significantly more effective than MI Paste Plus. MI Paste Plus and Clinpro Tooth Crème were found to be similar in their remineralising effect. It is worth noting that the fluoride concentration in Clinpro Tooth Cream (950 ppm F) is much lower than in Clinpro 5000 (5000 ppm F).

When its remineralising potential was compared *in vitro* to MI Paste Plus, Clinpro 5000 was found to be as effective as MI Paste Plus in remineralising enamel lesions and their effect was significantly greater than the effect of saliva (Chapla et al., 2013). The results of these previously mentioned studies were different due to difference in the study designs, duration of application of test products, and duration of the study.

### **1.3. Model Systems Used to Study Caries Process in Enamel**

The caries process can be studied and observed using different models, such as *in vitro*, *in situ*, animal, and randomised controlled clinical trials. Each model has its advantages and disadvantages. Among all, the randomised controlled clinical trial is considered the gold standard, though it is both time consuming and expensive.

#### **1.3.1. *In Vitro* Models**

The *in vitro* model is the most commonly used model in cariology research. It is a laboratory-based approach in which teeth preparation, caries lesion formation, and application of test materials are all carried out in the laboratory setting. In contrast to *in vivo* models, *in vitro* models can be performed with less cost, shorter time and they avoid participant compliance issues (Higham et al., 2005). Also by using these models only small sample sizes are required and the experimental environment can be highly controlled resulting in low variability (Buzalaf et al., 2010).

Among *in vitro* protocols, pH cycling models involve exposure of the dental tissue being either enamel or dentine obtained from human or bovine teeth with artificial caries lesions to demineralising and remineralising solutions with intermediary treatment with test products in order to mimic the dynamics of mineral loss and gain involved in the caries lesion. The pH-cycling models that are used widely were first introduced by ten Cate and Duijsters (1982). These models have been broadly used to evaluate the effectiveness of fluoridated dentifrices. The artificial caries lesions are created by immersion

of the substrates in buffered lactate or acetate solutions with a low pH ranging between 4.4 and 5.0, for times ranging between 16 hours and 28 days (White, 1987). Different types of lesions can be formed by this protocol such as surface softened lesions, also known as erosion-like lesions, or subsurface lesions, also called caries-like lesions (Buzalaf et al., 2010).

Still, these models cannot completely simulate the oral environment, and they also fail to mimic saliva/plaque fluid composition and the clearance of different products from the oral cavity as compared with *in vivo* conditions (Buzalaf et al., 2010).

### **1.3.2. Animal models**

Animal models were developed in the 1940s and they remarkably aid in the understanding of the real caries process and cavitation which occur under *in vivo* conditions. These models are very useful tools to simulate the natural progression of caries under true biological conditions such as the presence of saliva, presence of host defence components, and the natural clearance of test products. Compared with *in situ* caries models and *in vitro* caries models, animal models are the closest caries models (Marsh, 1995). However, this model has been criticised due to its irrelevancy to the real human caries process due to the fact that animals and humans differ significantly in their oral flora composition, eating habits, and morphology of the teeth (Higham et al., 2005; Stookey et al., 1995).

### 1.3.3. *In Situ* Models

*In situ* models can also be used to study the caries process. The first model was introduced by Koulourides et al. in 1974. The models involve the use of removable or fixed Intra-oral appliances designed to carry tooth tissue either enamel or dentine with caries lesions into a participant's mouth. This model, in fact, acts as a bridge between the highly controlled laboratory situation and the natural uncontrolled situation. This model is highly variable as there are many different variables that should be controlled and standardised to improve the power of the study such as hard tissue, diet, biofilm formation, and intra-oral site.

In contrast to *in vitro* studies, *In situ* models are used in the human mouth which ensures the simulation of the natural process of caries without causing any irreversible damage to the natural teeth and this is considered a major advantage. Other advantages are the flexibility of the study design and the control it has over the experimental variables. Compared to clinical trials, most of the associated ethical problems are avoided in the *in situ* model due to the fact that less time is needed to acquire the results. However, the outcome of studies using this model is influenced by the subjects' compliance (Cochrane et al., 2012; Zero, 1995; Damato and Stephen, 1994). These models are complicated by variations among participants in regard with dietary eating habits, salivary flow, buffer capacity, plaque compositions, thickness, and microbial composition all of which will affect the outcome of such models (Marsh, 1995).

## **1.4. Methods Used for Detecting Caries Lesion**

Caries lesions are primarily detected by a visual assessment and radiographic examination. These methods are considered as the conventional methods in detecting dental caries; however, they fail to detect caries lesions at an early stage. Therefore, the need to develop more advanced technologies capable of detecting such lesions has emerged.

The literature has described different tools that are nowadays used for detecting dental caries (Diniz et al., 2012).

### **1.4.1. Caries detection based on visual examination**

The caries process results in an alteration of surface characteristics of the hard dental structures that can be clinically visualised as opacities, or change in integrity, texture, and colour. Traditionally, dental caries is diagnosed by the help of a dental mirror, a sharp probe, a 3-in-1 syringe, a good source of light, and a dry tooth surface (Hamilton, 2005). However, sharp probes have been regarded as inappropriate tools for assessing dental lesions because they can irreversibly damage the enamel and transfer cariogenic bacteria from one tooth surface to another. Therefore, the ball-ended probes which are safer are recommended instead (Pitts, 2001, Stookey, 2005).

#### **1.4.1.1. International Caries Detection and Assessment System (ICDAS)**

The international Caries Detection and Assessment System (ICDAS) is a tool that is used to diagnose the extent and the activity of carious lesions. The different tooth surfaces are visually assessed and based on specific clinical



criteria can be categorised into different stages of caries (Pitts and Ekstrand, 2013).

This system was developed to try to provide clinicians with an evidence-based system for caries detection (Pitts, 2004). It is now available as ICDAS II and the tooth coronal caries criteria are as follows:

Code 0: Sound tooth surface

Code 1: First visual change in enamel obvious after prolonged air-drying or seen within the pit or fissure

Code 2: Distinct visual change in enamel

Code 3: Localised enamel breakdown without clinical signs of dentinal involvement

Code 4: Underlying dark shadow from dentine

Code 5: Distinct cavity with visible dentine

Code 6: Extensive distinct cavity with visible dentine

The ICDAS system is easy to be implemented in clinical practice and dental schools as it is a useful tool with acceptable sensitivity and specificity.

#### **1.4.2. X-Ray based Methods**

Radiography either conventional or digital is the most commonly used caries diagnostic tool in dentistry. Posterior bitewing radiography is considered as the main tool used for detecting approximal caries. In the case of occlusal caries, it can be recommended as a supplementary tool for detecting these lesions. This can be attributed to the low sensitivity of the bitewing to detecting occlusal caries as the literature has reported that occlusal caries

involving the upper third of the dentine may still be undetected on radiographs (Ricketts et al., 1995).

Radiographs are a useful tool to monitor caries progression or regression when taken at different points in time depending on the patient caries risk assessment. However, they are unable to differentiate between active, arrested, cavitated, and non-cavitated lesions. On the other hand, it is worth mentioning that bitewings are able to reveal hidden caries lesions otherwise undetectable by clinical examination (Ricketts et al., 1997).

### **1.4.3. Light-Emitting Devices**

Currently a number of caries assessment technologies have been developed that rely on optical properties of dental hard tissues. Some based on the fluorescence phenomenon of hard dental tissues which have been studied since for a long time. Fluorescence is a phenomenon by which an object is excited by a particular wavelength of light and the fluorescent (reflected) light is of a larger wavelength. When the excitation light is in the visible spectrum, the fluorescence will be of a different colour (Pretty, 2006). The enamel auto-fluorescence was first described by Benedict (1929) who suggested its use in the detection of dental caries. Research revealed that collagen crosslinked with the hydroxyapatite considered as the main fluorescing compound within the dental tissue (Lee et al., 2005).

The demineralisation of enamel was found to result in a reduction of its auto-fluorescence. In a caries lesion, the light scattering is much stronger than that in sound enamel (Ten Bosch, 1996) and the absorption of light per unit of volume is smaller, as a result, the fluorescence is less strong in the caries

lesion. The difference between the fluorescence of sound tissue and carious tissues can be quantified using different light-emitting devices such as Laser Fluorescence devices, Fibre Optic Trans-Illumination, and Quantitative light-induced fluorescence (Diniz et al., 2012).

#### **1.4.3.1. Laser Fluorescence Devices (DIAGNOdent and DIAGNOdent pen)**

Laser Fluorescence devices have been used as a non-invasive method for the detection of early carious lesions. These devices are capable of quantifying fluorescence emitted from different hard dental tissues when excited by a 655nm laser diode and they have shown good results in detecting occlusal caries. However, they should not be used as the only method for caries detection, but as an adjunct to both visual and radiographic examination (Diniz et al., 2012).

#### **1.4.3.2. Quantitative Light-induced Fluorescence**

Quantitative Light-induced Fluorescence (QLF) is a method initially developed for the detection of early caries lesions. It is based on the auto-fluorescence of dental hard tissue. When sound dental tissues are illuminated with high intensity blue light in the range of 370–500 nm they will start to emit light in the green part of the spectrum (Kuhnisch and Heinrich-Weltzien, 2004). Superficial demineralised tissue, however, results in light-scattering which means less light will reach the sound tooth surface underneath the lesion and as a result less tooth fluorescence will occur and this is indicated by loss of observed green fluorescence. Therefore, caries lesions appear as dark areas surrounded by bright green fluorescing healthy

tissue (Heinrich-Weltzien et al., 2003). Advanced caries lesions may also display some red fluorescence. This red fluorescence can also be observed in dental plaque and calculus (Buchalla, 2005).

The QLF device consists of a light source (blue light), a micro-camera used to capture the image of interest and quantifies the degree of fluorescence, and specific software to analyse the data and calculate the size, depth, and volume of the carious lesion (Pretty, 2006).

Sensitivity of QLF in detecting early caries lesions compared to visual examination has been assessed in many *in vitro* and *in vivo* trials. Pretty et al. (2002) in her *in vitro* study showed that QLF was successfully capable of detecting demineralised primary smooth tooth surfaces that were not clinically visible.

An *in vivo* study conducted by Kuhnisch et al. (2007) found QLF to be as effective as visual examination in detecting 78.8% of the non-cavitated occlusal lesions. However, QLF was significantly more sensitive than visual examination in detecting smaller lesions.

#### **1.4.3.3. Fibre-Optic and Digital Imaging Fibre-Optic Trans-Illumination**

Fibre optic trans-illumination is based on the variation in light-scattering activity between sound and carious tooth surfaces. The method involves a high-intensity light source and a probe tip of appropriate size. A carious lesion can be detected by applying light on the smooth or interproximal surface of the tooth and assessing the tooth surface carefully from the opposite or the occlusal side. As demineralised tissues scatter more light, they appear dark on a light background. However this method can be subject to high inter and intra-examiner variation because it solely relies on the eye

to diagnose caries lesions (Neuhaus et al., 2009). Research found that the fibre optic trans-illumination technique increased the detection of approximal carious lesions (Davies et al., 2001). However, when compared with visual examination and bitewing radiographs, it was found to be the least reliable tool in detecting approximal caries (Hintze and Wenzel, 1998).

Fibre optic trans-illumination technique has been developed further into Digital Imaging Fibre Optic Trans-Illumination that is capable of capturing an image of the lesion and viewing it on the computer screen. Research found that this technique presents higher sensitivity in detecting early lesions compared to radiographic examination, although it only can detect the presence of caries lesions but not the size or extent of them (Young & Featherstone, 2005). Schneiderman et al. in 1997 evaluated the accuracy of digital imaging fibre optic trans-illumination as a tool for detecting approximal, occlusal and root caries and found high sensitivities for caries detection but lower sensitivities when compared with radiographic images and examination. There are several limitations of this diagnostic tool. This method does not measure lesion depth and has lower specificities when compared with conventional radiographs which means over-diagnosis (Young, 2002).

## 1.5. Research Aims and Hypotheses

### 1.5.1. Aims

1. To investigate *in vitro* the effect of Calcium Phosphate delivering technologies: Clinpro 5000 (Tri-Calcium Phosphate, 5000 ppm F) and Clinpro Tooth Crème (Tri-Calcium Phosphate, 950 ppm F) in the remineralisation of artificial enamel subsurface lesions of bovine teeth using QLF technology.
2. To compare calcium phosphate delivering technologies remineralising potential with 5000 ppm F, 1450 ppm F toothpastes, and 0 ppm F toothpaste as a negative control using QLF technology.
3. To compare the remineralising capacity of CPP-ACP, with and without fluoride (Tooth Mousse – 10% w/v CPP-ACP and MI Paste Plus – 10% w/v CPP-ACP, 900 ppm F) when used supplementary to 1450 ppm F toothpaste on artificial early enamel subsurface lesions of bovine teeth using QLF technology.

### 1.5.2. Null hypotheses

1. There are no statistically significant differences in the enamel remineralisation that result from 1450 ppm F toothpaste + Tooth Mousse, 1450 ppm F toothpaste + MI Paste Plus, Clinpro 5000, Clinpro Tooth Crème, 1450 ppm F toothpaste, and 5000 ppm F toothpaste.
2. There are no statistically significant difference in the enamel remineralisation that results from CPP-ACP with and without fluoride when used supplementary to 1450 ppm F toothpaste.

## Chapter 2

### MATERIALS AND METHODS

This *in vitro* study was carried out to assess the remineralising potential of different calcium phosphate delivering materials in comparison with fluoride products, using QLF in a pH-cycling model simulating oral cavity conditions. The protocol followed in the current study will be discussed in this section.

#### 2.1. Power Calculation

Statistical advice was sought and the sample size was calculated by using data from a previous study of Bataineh et al. (2017). The standard deviation of the response variable was assumed to be 2.57 with 95% power and 0.05 significance level. The true difference between treatments was calculated to be 2.572 and it was determined that a total of 26 enamel slabs per group were needed.

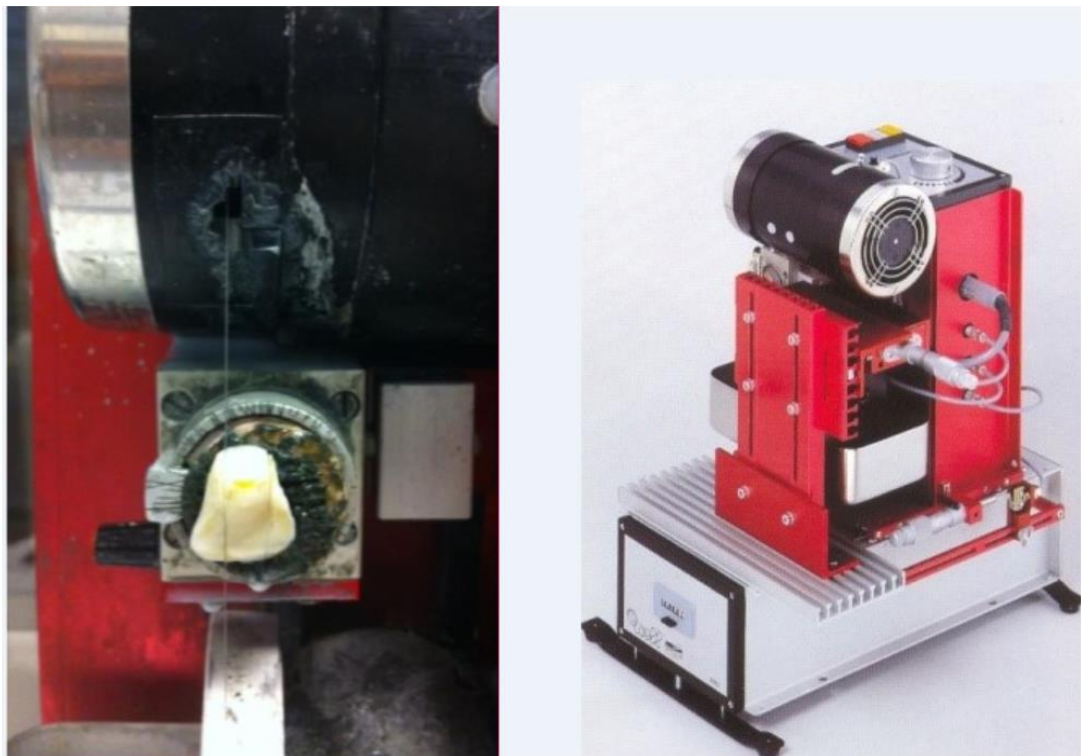
#### 2.2. Preparation of enamel slabs

Bovine teeth were used in this study after obtaining the Food Standards Agency approval (Appendix 1). All enamel slabs were created from bovine lower incisors which were immersed in 12% sodium hypochlorite for 24 hours to eliminate prions. A previous study showed that sodium hypochlorite did not have an effect on the mineral content of dentine or its crystal structure



(Driscoll et al., 2002). Then teeth had been stored in distilled water and 0.1% thymol (Sigma Aldrich, thymol 98%) at room temperature. Teeth were debrided of any soft tissue before their sectioning using a spoon excavator and screened by transillumination and transmitted light using low-power microscopy (Leitz, Wetzlar®, Germany) to detect any cracks, caries or malformations. In order to remove pellicles and make the enamel surface flat, included teeth were lightly abraded with fine abrasive paper.

Green stick impression compound (Kerr, UK) was used to mount teeth on plates. Following this, crowns were sectioned using a water cooled, diamond wire saw, and cutting machine (Well@Walter Ebner, CH-2400 Le Loche). Each crown was sectioned into buccal and lingual parts. Approximately 4mm x 4 mm x 2mm size slabs were prepared from the buccal surfaces.



**Figure 1: Diamond wire saw apparatus used for teeth sectioning (Well® Walter EBNER, CH-2400 Le Loche)**

Each enamel slab was mounted on a plastic spoon of a 'Sterilin'-type universal tube by sticky wax to hold the slab in the demineralising gel. The plastic spoon was secured to the screw cap of the universal tube keeping the slab suspended in the centre of the tube free space (Figure 2).



**Figure 2: Enamel slab attached to the plastic spoon with sticky wax and suspended in the plastic universal tube**

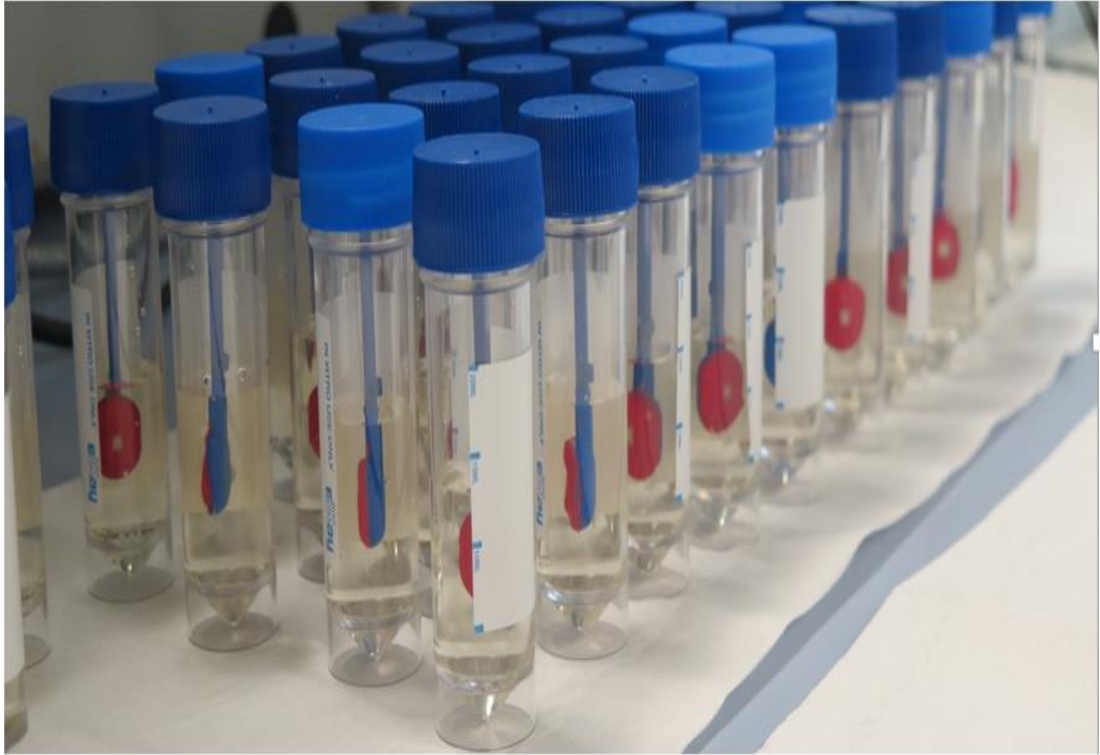
A coloured nail varnish (Max Factor "Glossfinity") with acid resistance properties was then applied to the whole surface of enamel slabs leaving a small window of approximately 2 x 3 mm in the centre of each slab representing the lesion

area (Figure 3). A second layer of the same nail varnish was applied after 24 hours of drying of the first layer.



**Figure 3: Enamel slab covered with nail varnish except for a small window**

In order to keep moisture and prevent slabs dehydrating, enamel slabs were stored in the plastic universal tubes containing deionised distilled water at room temperature once they were prepared (Figure 4).



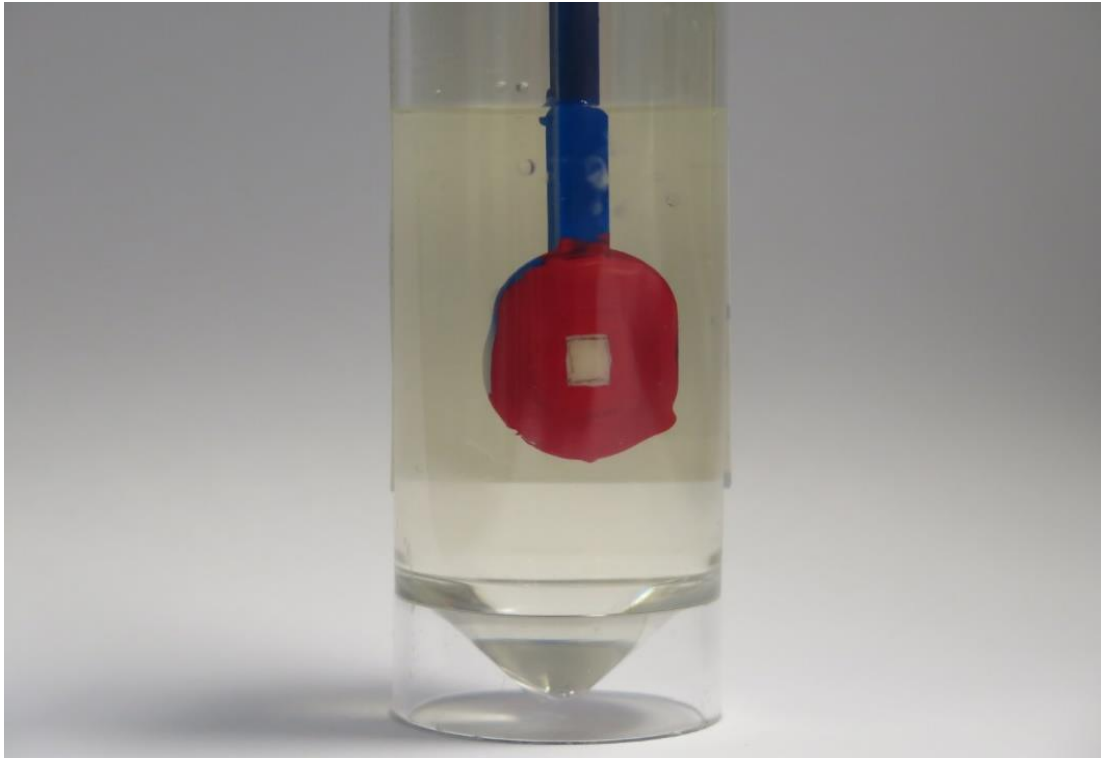
**Figure 4: Enamel slabs stored in plastic universal tubes**

### **2.3. Preparation of Enamel Subsurface lesions**

In order to create subsurface caries-like lesions, a demineralising medium of an acidified hydroxyethyl cellulose gel was used. The gel consisted of 0.1 M lactic acid (Sigma Aldrich D/L GPR 87% Lactic acid) stirred to a thick consistency with 6% w/v hydroxyethyl cellulose (Sigma Aldrich). The pH value of the gel was adjusted to 4.5 by the addition of 0.1 M sodium hydroxide (BDH Analar Grade). The mixture was left to settle for 24 hours. Once the demineralising gel was ready for use, it was poured into the universal tubes.

Artificial enamel subsurface lesions were created by immersing all enamel slabs in acid gel for 10 days (Figure 5). The enamel slabs were then removed from the acid gel and washed with distilled water to remove any residual adsorbed gel from the enamel surface. Before taking the baseline QLF

measurements, the nail varnish was removed using methanol. This step was carried out for all the enamel slabs before their random allocation to the treatment groups.



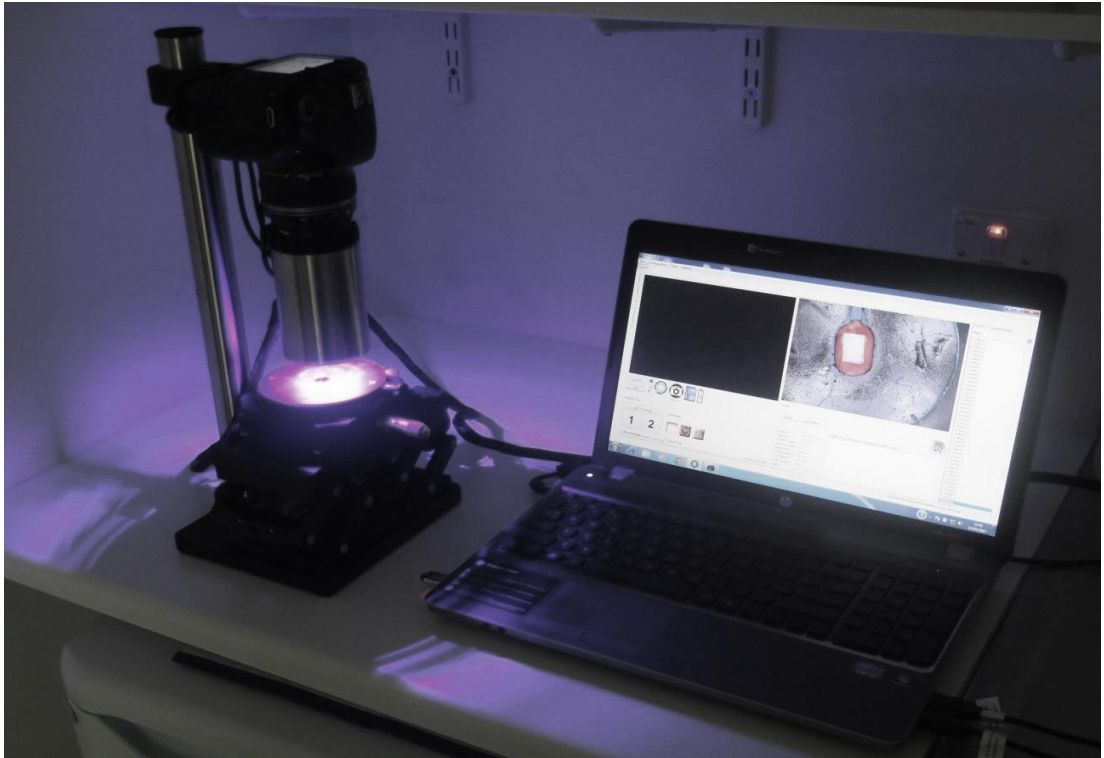
**Figure 5: Enamel Slab immersed in acidified hydroxyethyl cellulose gel for 10 days to create a subsurface caries-like lesion**

#### **2.4. Quantitative Light-induced Fluorescence imagery**

In the present *in vitro* study, two QLF measurements were taken for each enamel slab using the QLF system (QLF-D Biluminator™ 2 Inspektor Research Systems BV, Amsterdam, The Netherlands). The first measurement was taken before the pH-Cycling after the creation of the artificial enamel subsurface lesions whereas the second measurement was taken at the end of the pH-Cycling which extended for 28 days.

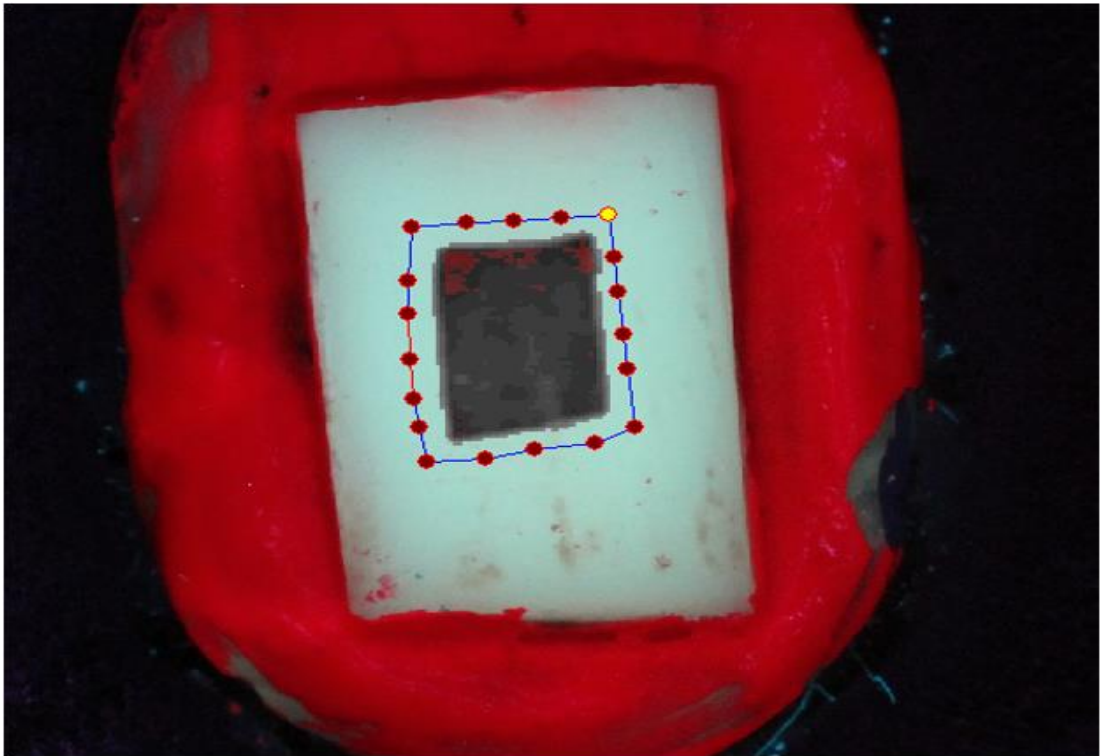
The QLF-D Biluminator™ 2 which consists of a Biluminator™ was mounted on a Single Lens Reflex (SLR) camera fitted with a 60 mm macro lens. The Biluminator™ provides the light sources and filters for making white-light and QLF™-images. Fluorescence images of all enamel specimens were captured with a 'Live 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 operator.

In order to ensure that all QLF images were captured at the same position from the same angles under optimum illumination, the QLF camera was secured in place all the time by using a stand (Figure 6). Specimen stability, light intensity, and magnification were controlled using the jig in order to standardise the camera-specimen distance.



**Figure 6: QLF machine and camera fixed in position on a stand**

A patch was drawn around each white spot lesion area by the study examiner with its borders on sound enamel (Figure 7). Inside this patch, the fluorescence levels of sound tissue were reconstructed by using the fluorescence radiance of the surrounding sound enamel. The percentage difference between the reconstructed and the original fluorescence levels was calculated. The same area of interest was used for the baseline and endpoint white spot lesion image identification.



**Figure 7: QLF image taken with the blue light showing a patch drawn in sound enamel around the lesion with the demineralised lesion in the centre of the enamel slab**

The artificial caries lesion (white spot lesion) appeared as dark area. The fluorescent radiance of the white spot lesion viewed by QLF was lower than that of the surrounding sound enamel. In order to enable calculation of loss of fluorescence in the white spot lesion, the fluorescence radiance of sound tissue at the lesion site was reconstructed by interpolation from the radiance of the sound tissue surrounding the lesion. Fluorescence radiance levels less than 95% of reconstructed sound fluorescence radiance levels were considered to be artificial early caries lesions and were displayed as shades of grey where darker grey corresponded to higher fluorescence loss. The difference between the measured values and the reconstructed values gave the resulting fluorescence loss in the lesion.



For each enamel lesion the following three metrics were obtained:

1.  $\Delta F$ : Percentage fluorescence loss with respect to the fluorescence of sound tooth tissue; related to lesion depth (%). It could be said that the greater the lesion depth, the more disordered the structure of the enamel prisms became, thereby resulting in increased light absorption and scattering in the enamel lesion (Ando et al., 2001; Shi et al., 2001). Research has shown a good correlation between  $\Delta F$  value and the demineralisation depth (Nakata et al., 2009).
2. Area: The surface area of the lesion expressed in pixels<sup>2</sup> (px<sup>2</sup>).
3.  $\Delta Q$ : The lesion area multiplied by the mean change in fluorescence radiance. Related to lesion volume (% px<sup>2</sup>). Changes in lesion area and fluorescence radiance loss together give a measure of the changes in lesion severity.

## **2.5. $\Delta F$ range of the artificial lesions**

After performing the QLF baseline analysis for all enamel slabs, the range of  $\Delta F$  values were found to vary between -11.16 and -44.3. The enamel slabs with the  $\Delta F$  range (-24.6 to -36.2 with an average of -30.4) were selected for the experiment in order to pick up the differences in  $\Delta F$  after treatment.

## 2.6. Experimental and control groups

The enamel slabs were randomly assigned to seven groups as follows:

**1. Group 1: 0 ppm F toothpaste (Negative Control)**

0 ppm F toothpaste was used twice daily before and after the pH cycling regimen.

**2. Group 2: 1450 ppm F Toothpaste (Positive Control)**

1450 ppm F toothpaste was applied twice daily before and after the pH cycling regimen.

**3. Group 3: 5000 ppm Fluoride Toothpaste**

5000 ppm F toothpaste was applied twice a day before and after the pH cycling regimen.

**4. Group 4: Tooth Mousse (10% w/v CPP-ACP) Plus 1450 ppm F toothpaste**

1450 ppm F toothpaste was applied twice daily before and after the pH cycling regimen, and Tooth Mousse was applied once a day. Following the last application of 1450 ppm F toothpaste, the enamel slabs were gently washed with distilled water and were placed in the Tooth Mousse slurry and then in night time saliva.

**5. Group 5: MI Paste Plus (10% w/v CPP-ACP + 900 ppm F) Plus 1450 ppm F toothpaste**

1450 F toothpaste was applied twice daily before and after the pH cycling regimen, and MI paste plus was applied once a day. Following the last application of 1450 ppm F toothpaste, the enamel slabs were

gently washed with distilled water and were placed in the MI paste plus slurry and then in night time saliva.

**6. Group 6: Clinpro 5000 (Tri-Calcium Phosphate, 5000 ppm F)**

Clinpro 5000 was applied twice a day before and after the pH cycling regimen.

**7. Group 7: Clinpro Tooth Crème (Tri-Calcium Phosphate, 950 ppm F)**

Clinpro Tooth Crème was applied twice daily before and after the pH cycling regimen.

## **2.7. Experimental Materials**

The experimental pastes were used as slurries and were used according to the manufacturer instruction. The enamel slabs were immersed in the experiment solutions for 30 minutes before and after demineralisation cycle.

The study materials were as follows:

1. Non-Fluoride toothpaste (the Boots Company PLC, Nottingham, England) which was used as a negative control in order to investigate the effect of artificial saliva in remineralising early enamel subsurface lesions.



**Figure 8: Fluoride-free toothpaste (0 ppm fluoride)- Boots Smile Non Fluoride**

2. Fluoride toothpaste 1450 ppm F (0.32% w/w sodium fluoride) (Colgate cool stripe. Colgate–Palmolive (UK) Ltd, Guildford, England). This is the maximum permissible concentration over the counter in the United Kingdom.



**Figure 9: Sodium Fluoride toothpaste 1450 ppm – Colgate Cool Stripe cavity protection**

3. High fluoride toothpaste 5000 ppm F (Sodium fluoride 1.1% w/w 5000 ppm F, Duraphat®. Colgate–Palmolive (UK) Ltd, Guildford, England). This concentration is only prescribed by dental profession for high caries risk individuals.



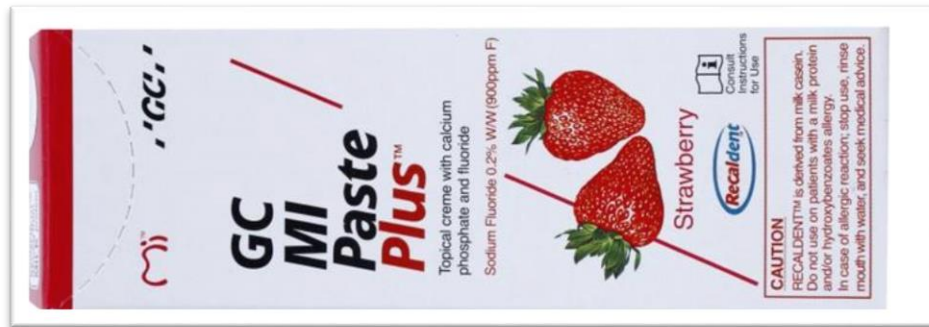
**Figure 10: Duraphat Toothpaste 5000 ppm Fluoride as Sodium Fluoride – Colgate Duraphat 1.1% W/W**

4. Tooth Mousse 10% w/v CPP-ACP (GC Tooth Mousse™, GC Corp. Tokyo, Japan).



**Figure 11: GC Tooth Mousse – Topical crème with bio-available calcium and phosphate – 10% w/v CPP-ACP**

5. MI Paste Plus 10% w/v CPP-ACP, 900 ppm F (0.2% w/w sodium fluoride) (GC MI Paste Plus™, GC Corp, Tokyo, Japan).



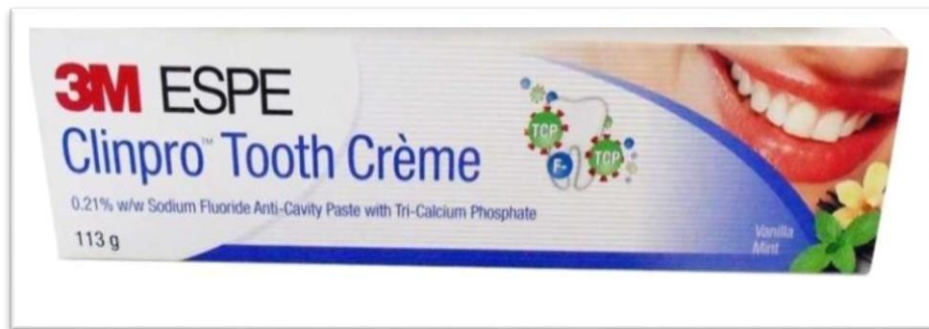
**Figure 12: GC MI Paste Plus with 900 ppm Fluoride – Topical crème with calcium phosphate and fluoride – 10% CPP-ACP & 0.2% w/w sodium fluoride)**

6. Clinpro™ 5000: Tri-Calcium Phosphate, 5000 ppm F Toothpaste (1.1% Sodium Fluoride) (3M ESPE dental, United Kingdom).



**Figure 13: Clinpro 5000 – Tri-Calcium Phosphate with 5000 ppm Fluoride – 1.1% Sodium Fluoride (3M ESPE dental, United Kingdom)**

7. Clinpro™ Tooth Crème: Tri-Calcium Phosphate, 950 ppm F Toothpaste (0.21% Sodium Fluoride) (3M ESPE dental, United Kingdom).



**Figure 14: Clinpro Tooth Crème – Tri-Calcium Phosphate with 950 ppm Fluoride – 0.21% Sodium Fluoride (3M ESPE dental, United Kingdom)**

## 2.8. Toothpaste slurry

Toothpaste slurries were prepared by mixing the toothpaste with artificial day time saliva in a volume ratio 1:4 (toothpaste: saliva) by weight, using a WhirliMixer® (Fisons) for 1 minute.

## 2.9. Randomisation and Blinding

Enamel slabs were randomly allocated to seven groups using a table of random numbers, according to a computer programme of random allocation. During QLF imaging and analysis, the study investigator was blinded and did not know to which group the enamel slab belonged.

## 2.10. The pH Cycling Regime

A total of 28 enamel slabs with artificial early enamel subsurface lesions were used per group two more than determined by power calculation in case any slabs were damaged or lost during the study. The enamel slabs were randomly assigned to the seven groups with seven different remineralising materials. Enamel slabs were cycled for 28 days to allow sufficient time for the test products to show their effectiveness. At the start of each day, slabs were rinsed with distilled water for 1 minute and dipped in toothpaste slurry for 30 minutes. After that enamel slabs were rinsed with distilled water for 1 minute and placed in day time artificial saliva for 90 minutes. Enamel slabs were then immersed in demineralisation solution (acetic acid solution with pH 4.8) for 5 minutes then they were rinsed with distilled water for 1 minute and suspended in day time artificial saliva for 90 minutes. This process was repeated 5 times a day. After the fifth dipping in the demineralisation solution the enamel slabs were dipped in toothpaste slurry for 30 minutes.

In 0 ppm F, 1450 ppm F and 5000 ppm F, Clinpro Tooth Crème, and Clinpro 5000 groups enamel slabs were then placed in the night time artificial saliva. While in 1450 ppm F + Tooth Mousse and 1450 ppm F + MI Paste Plus groups, following the toothpaste dipping, the enamel slabs were rinsed gently with distilled water and then dipped in the Tooth Mousse or MI Paste Plus slurries for 30 minutes and finally were placed in the night time artificial saliva.

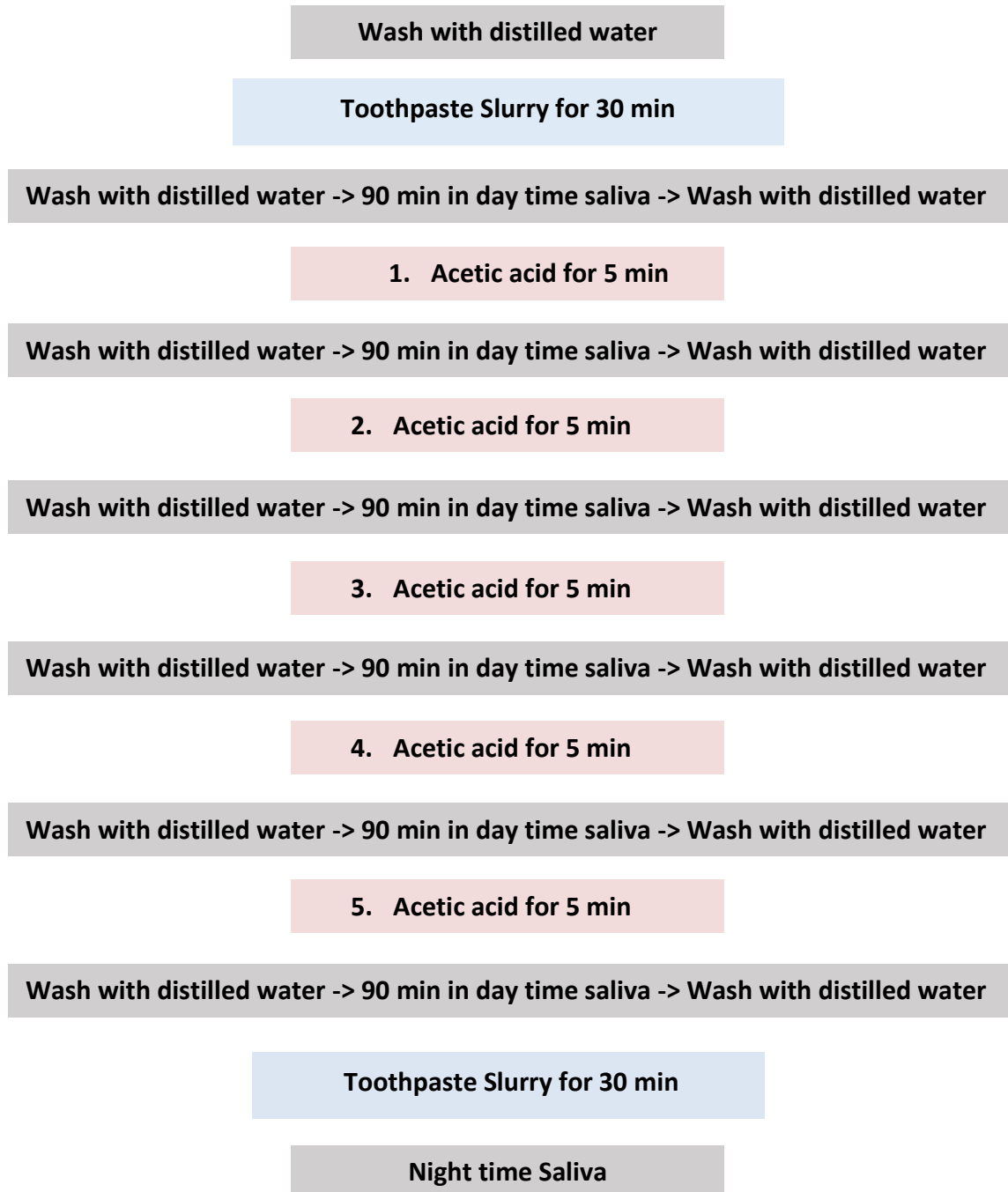
The day time artificial saliva was supersaturated with respect to hydroxyapatite in order to allow remineralisation of enamel slabs during the



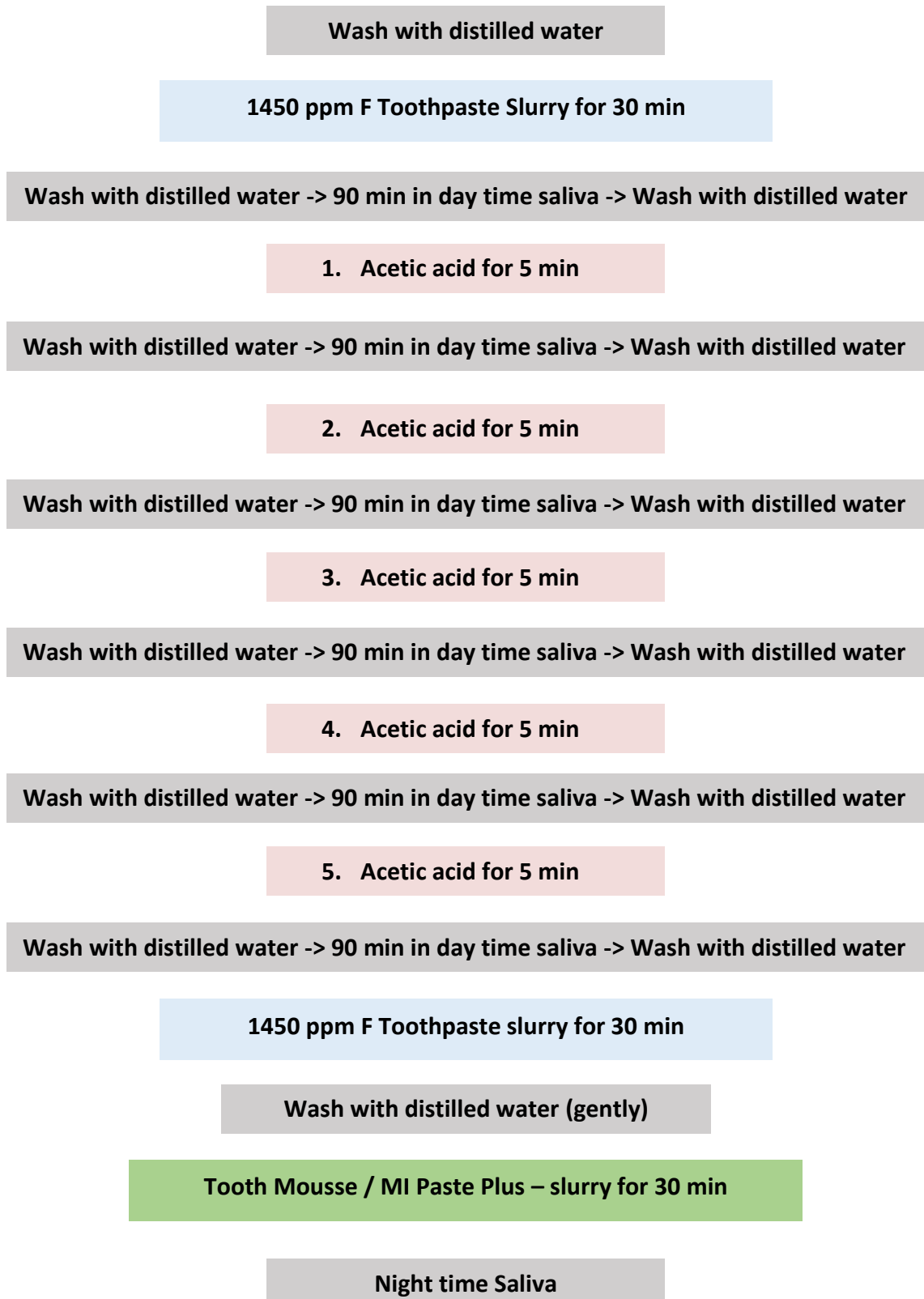
day, whereas the night time artificial saliva was saturated solution with respect to hydroxyapatite and it was used overnight in order to ensure that no further precipitation occurred on the slab surface; when the cycling was resumed the next morning, the slabs were in the same state of demineralisation and remineralisation. The acetic acid was changed after each exposure. Both day time and night time saliva were changed every day. The enamel slabs were kept in an incubator at 37°C at all times except during the dipping in the toothpaste slurry or the demineralisation solution.

## 2.11. Flow Charts

Figure 15: Flow chart for 0 ppm F, 1450 ppm F, 5000 ppm, Clinpro Tooth Crème, and Clinpro 5000 toothpastes



**Figure 16: Flow chart for 1450 ppm F + Tooth Mousse and 1450 ppm F + MI Paste Plus**



## **2.12. Preparation of solutions**

### **2.12.1. Artificial Saliva**

Two artificial saliva solutions were used in this study; day time and night time artificial saliva. The day time artificial saliva was used for day time during the pH cycling, between the acid exposures and was a supersaturated solution with respect to hydroxyapatite that allowed remineralisation of enamel slabs. The night time artificial saliva was used to store the slabs during the night and was a saturated solution with respect to hydroxyapatite that maintained the enamel condition and did not provide any minerals exchange.

The artificial saliva composition was based on the electrolyte composition of natural saliva and it was advised to be used in order to eliminate any precipitation on the enamel surface (as provided by Dr R. P. Shellis, Department of Oral and Dental Science, University of Bristol, Bristol, UK).

**2.12.1.1. The preparation of day time artificial saliva**

The composition of day time artificial saliva is shown in Table 1.

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

**Table 1: Composition of day time artificial saliva**

Other ingredients - 900 mL distilled water and 1.8 mL 1 mol/L HCL were added to the above components and all were stirred using a shaker until it dissolved. The pH was adjusted to 6.8 by adding KOH solution that was made up to 1L with de-ionised water.

**2.12.1.2. The preparation of night time artificial saliva**

The formulation of the night time artificial saliva is shown in Table 2.

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

**Table 2: Composition of night time artificial saliva**

Again, 900 mL distilled water 1.4 mL 1 mol/L HCL and above components were stirred using a shaker until it dissolved. The pH was adjusted to 6.8 by adding KOH solution that was made up to 1L with de-ionised water.

### 2.12.2. Acetic Acid Preparation

The preparation of acetic acid solution was done according to ten Cate *et al.* (2006). Table 3 shows the constitution of acetic acid solution.

Contents	Concentration g/L
Calcium chloride	1.66
Potassium di-hydrogen phosphate	1.22
Acetic acid	30.02

**Table 3: Composition of acetic acid solution**

The above contents and 1 L distilled water were centrifuged until fully dissolved. The pH was adjusted using a pH probe and pH meter (ORION-model 920A) by adding KOH solution to reach pH 4.8.

### 2.13. Training and calibration

Before the study, the study investigator received a training on how to use the QLF machine (QLF-D Biluminator™ 2) and how to use the software including image capturing and image analysis to obtain the QLF measurements (Inspektor Research Systems BV, Amsterdam, The Netherlands). The investigator assessment of sound, demineralised enamel and the borders of the lesion during image analysis were calibrated.

## **2.14. Intra-Examiner Reliability**

At the end of the study, QLF measurements of approximately 15% of enamel slabs were repeated in order to allow the assessment of intra-examiner reliability using the Intra-Class Correlation Coefficient (ICC).

## **2.15. Statistical Analysis of the Data**

SPSS statistical software package for windows version 22.0 was used to statistically analyse the data. The mean, median, range, and standard deviation were calculated.

The Shapiro-Wilk test and Kolmogorov-Smirnov test were used to assess the normality of the data distribution.

Paired sampled t-tests were performed to compare the changes in remineralisation from baseline and after treatment within each group.

Depending on the normality of the data, one way ANOVA or Kruskal-Wallis Tests were used to compare between the seven groups. Furthermore, the Bonferroni test was used to assess if there were any significant differences between the groups. The test also calculated the 95% confidence intervals. The significance level for all tests was set at  $P < 0.05$ .



## Chapter 3

### RESULTS

Three main parameters for QLF were statistically analysed, these were:

- $\Delta F$ : Percentage fluorescence loss with respect to the fluorescence of sound tooth tissue. Related to lesion depth (%).
- Area: The surface area of the lesion expressed in pixels<sup>2</sup> (px<sup>2</sup>).
- $\Delta Q$ :  $\Delta F$  times the Area. Percentage fluorescence loss with respect to the fluorescence of sound tissue times the area. This is related to lesion volume (%px<sup>2</sup>).

#### 3.1. The mean fluorescence loss $\Delta F$

To ensure that any significant differences found to exist between the groups at the end of the study are due to real differences in the remineralising potentials of the test materials, the values of  $\Delta F$ -Baseline for all groups were checked to see if there were differences between the groups. First of all, the normality of the data was checked using the normality test (Shapiro-Wilk test and Kolmogorov-Smirnov test). P values were larger than 0.05 and statistically not significant, thus the data was considered normally distributed (0.200 and 0.370 respectively). The ANOVA test was used to assess if there were any statistically significant differences in  $\Delta F$ -Baseline values between the lesions assigned to the seven groups. No statistically significant differences were found.

### **3.1.1. Difference in $\Delta F$ within each group**

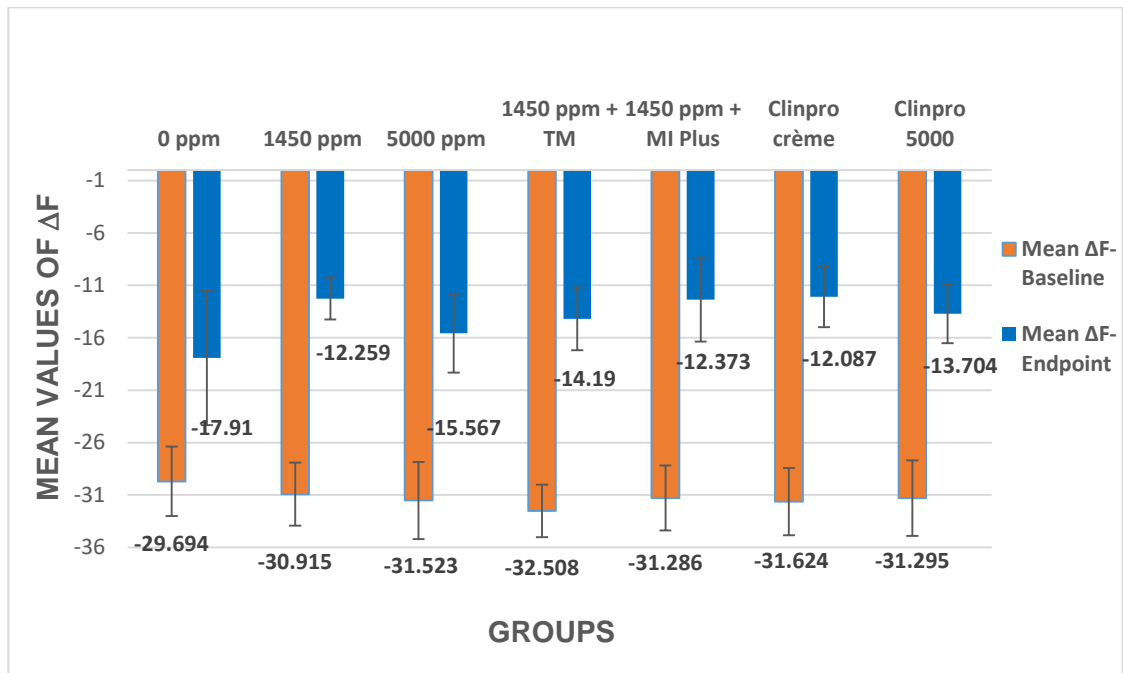
To assess whether the difference between  $\Delta F$ -Baseline and  $\Delta F$ -Endpoint was significantly different within the same group, paired T-Tests were used.

Table 4 shows the results of the paired T-Test and demonstrates a statistically significant improvement in the  $\Delta F$ -Endpoint compared with  $\Delta F$ -Baseline in all groups ( $P < 0.05$ ).

Figure 17 shows the change in the mean of  $\Delta F$ -Baseline and  $\Delta F$ -Endpoint with the standard deviation for all groups.

Groups	Mean $\Delta F$ - Baseline $\pm$ SD	Mean $\Delta F$ - Endpoint $\pm$ SD	Mean $\Delta F$ - Differen ce $\pm$ SD	95% CI of the Difference		Sig. (2- tailed)
				Lower	Upper	
0 ppm F	-29.69 $\pm$ 3.31	-17.91 $\pm$ 6.43	11.78 $\pm$ 5.86	9.41	14.14	0.000*
1450 ppm F	-30.91 $\pm$ 3.09	-12.26 $\pm$ 2.03	18.65 $\pm$ 2.98	17.45	19.9	0.000*
5000 ppm F	-31.52 $\pm$ 3.68	-15.57 $\pm$ 3.76	16.1 $\pm$ 3.96	14.45	17.65	0.000*
1450 ppm F + Tooth Mousse	-32.50 $\pm$ 2.54	-14.19 $\pm$ 3.06	18.31 $\pm$ 3.29	17	19.6	0.000*
1450 ppm F + MI Paste Plus	-31.28 $\pm$ 3.12	-12.37 $\pm$ 3.99	18.91 $\pm$ 4.44	17.1	20.7	0.000*
Clinpro Crème	-31.62 $\pm$ 3.16	-12.09 $\pm$ 2.86	19.53 $\pm$ 3.96	17.9	21.1	0.000*
Clinpro 5000	-31.29 $\pm$ 3.62	-13.70 $\pm$ 2.84	17.59 $\pm$ 3.59	16.1	19	0.000*

Table 4: Paired sampled T-Test results for values of  $\Delta F$ -Baseline and  $\Delta F$ -Endpoint



**Figure 17: Mean values of  $\Delta F$ - Baseline and  $\Delta F$ -Endpoint for all groups**

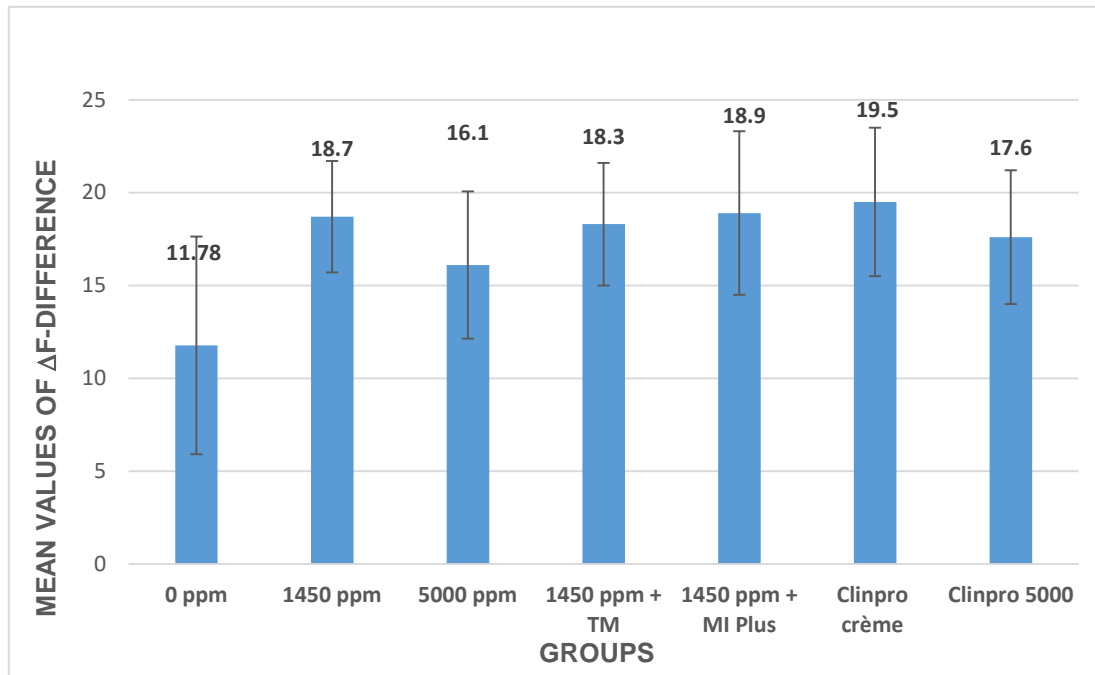
### 3.1.2. Difference in $\Delta F$ between all groups

The  $\Delta F$ -Difference (change) was calculated using the following formula:

$$\text{Difference in } \Delta F = (\Delta F \text{ after treatment}) - (\Delta F \text{ at Baseline})$$

Figure 18 shows the mean of  $\Delta F$ -Difference of all groups. The values of  $\Delta F$ -Difference in all groups were positive, indicating a decrease in  $\Delta F$  (mean fluorescence loss) after treatment compared to that at baseline. Clinpro Tooth Crème group showed the highest reduction in  $\Delta F$  ( $19.537 \pm 3.962$ ), followed by (1450 ppm F toothpaste + MI Paste Plus) group, 1450 ppm F toothpaste group, (1450 ppm F toothpaste + Tooth Mousse) group, Clinpro 5000 group at a mean difference of ( $18.913 \pm 4.447$ ), ( $18.656 \pm 2.984$ ), ( $18.318 \pm 3.291$ ), ( $17.591 \pm 3.591$ ) respectively. 0 ppm F toothpaste group

had the least amount of reduction in mean  $\Delta F$ -Difference ( $11.78 \pm 5.86$ ) followed by 5000 ppm F toothpaste group ( $16.058 \pm 3.957$ ).



**Figure 18: Means of the  $\Delta F$ -Difference of all groups**

Normality tests including Shapiro-Wilk test and Kolmogorov-Smirnov test were carried out to check if the  $\Delta F$ -Differences were normally distributed.

Data was not considered normally distributed as the P values were statistically significant ( $P > 0.05$ ) and hence non-parametric tests were used.

In order to assess if the  $\Delta F$ -Difference was statistically significant between the seven groups, the Kruskal-Wallis test was performed. The result showed that the mean of  $\Delta F$ -Difference was statistically significant between the seven groups ( $P < 0.05$ ). In order to find out which groups were statistically different pairwise comparisons were performed.

Table 5 shows the result of pairwise comparison tests with the Bonferroni correction which corrects for multiple testing. This test was conducted in order to determine which inter-group comparisons were significantly different. It can be seen that the mean of  $\Delta F$ -Difference of the 0 ppm F toothpaste group is statistically significantly lower than the mean of  $\Delta F$ -Difference in all groups except the 5000 ppm F toothpaste group and Clinpro 5000 group. The mean  $\Delta F$ -Difference of the Clinpro Tooth Crème group is statistically significantly higher than the mean of  $\Delta F$ -Difference of 5000 ppm F toothpaste group.

	Test statisti c	Std. Error	Std. Test Stat	Sig.	Adj. Sig
0 ppm F – Clinpro Tooth Crème	-70.96	14.61	-4.85	0.00	0.000
0 ppm F – 1450 ppm F	-60.30	14.61	-4.12	0.00	0.001
0 ppm F - (1450 ppm F +Tooth Mousse)	-59.48	14.61	-4.07	0.00	0.001
0 ppm F – (1450 ppm F + MI Paste Plus)	-58.92	14.61	-4.03	0.00	0.001
5000 ppm F – Clinpro Tooth Crème	-48.76	14.61	-3.33	0.00	0.018
0 ppm F – Clinpro 5000	-43.67	14.61	-2.98	0.00	0.059
5000 ppm F - (1450 ppm F + Tooth Mousse)	-37.28	14.61	-2.55	0.01	0.225
5000 ppm F – (1450 ppm F + MI Paste Plus)	-36.73	14.61	-2.51	0.01	0.251
0 ppm F – 5000 ppm F	-22.19	14.61	-1.51	0.12	1.000
5000 ppm F – Clinpro 5000	-21.48	14.61	-1.47	0.14	1.000
(1450 ppm F + MI Paste Plus) – Clinpro Tooth Crème	-12.03	14.61	-0.82	0.41	1.000
(1450 ppm F + Tooth Mousse) – Clinpro Tooth Crème	-11.48	14.61	-0.78	0.43	1.000
1450 ppm F – Clinpro Tooth Crème	-10.65	14.61	-0.72	0.46	1.000
(1450 ppm F + MI Paste Plus) – (1450 ppm F + TM)	0.55	14.61	0.03	0.97	1.000
(1450 ppm F + Tooth Mousse) – 1450 ppm F	0.82	14.61	0.05	0.95	1.000
(1450 ppm F + MI Paste Plus) – 1450 ppm F	1.38	14.61	0.09	0.92	1.000
Clinpro 5000 – (1450 ppm F + MI Paste Plus)	15.25	14.61	1.04	0.29	1.000
Clinpro 5000 - (1450 ppm F + Tooth Mousse)	15.80	14.61	1.08	0.27	1.000
Clinpro 5000 - 1450 ppm F	16.63	14.61	1.13	0.25	1.000
Clinpro 5000 - Clinpro Tooth Crème	27.28	14.61	1.86	0.06	1.000
5000 ppm F - 1450 ppm F	38.11	14.61	2.60	0.00	0.191

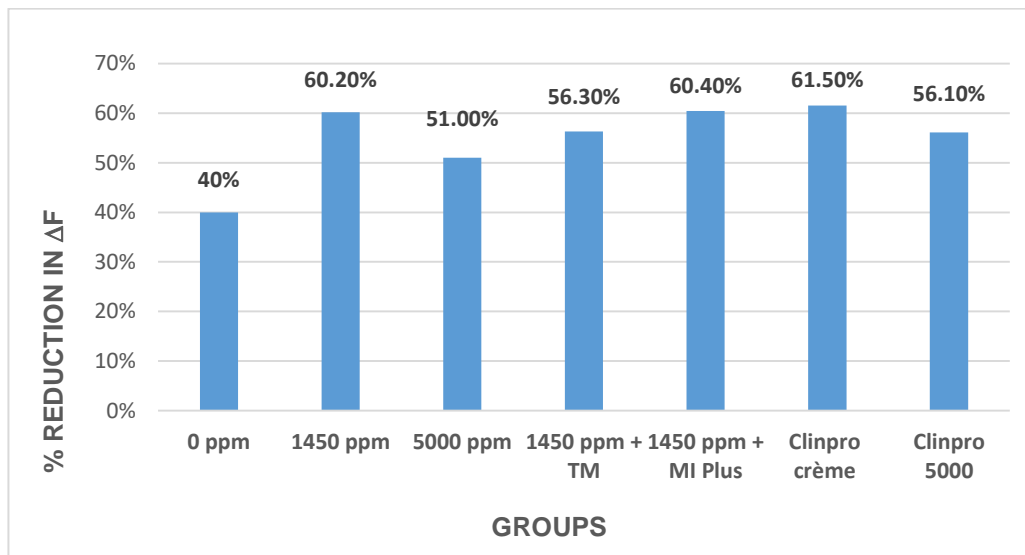
**Table 5: Multiple comparisons of  $\Delta F$ -Differences between groups with Bonferroni correction**

### 3.1.3. The percentage reduction in $\Delta F$

The percentage of reduction in  $\Delta F$  ( $\% \Delta F$ ) was calculated using the following formula:

$$(\Delta F\text{-Difference} / \Delta F\text{-Baseline}) \times 100$$

Figure 19 shows the % change in  $\Delta F$  values for all groups. It was highest in the Clinpro Tooth Crème group at 61.8% and lowest in the 0 ppm F toothpaste group at 40%



**Figure 19: The % reduction in  $\Delta F$  values for all groups**

### 3.1.4. Intra-examiner reproducibility for $\Delta F$

The intra-examiner reproducibility was tested using the intra-class correlation coefficient. A random selection of 28 enamel slabs which accounts for 15% of the enamel slabs was carried out. These 28 enamel slabs were re-analysed. The Intra-class Correlation Coefficient was found to be (0.99) which represents excellent reproducibility.



### **3.2. The mean $\Delta Q$ : Lesion volume**

Normality tests (Shapiro-Wilk test and Kolmogorov-Smirnov test) were carried out for the  $\Delta Q$ -Baseline values and showed that the data were normally distributed for all groups. Therefore, a one way ANOVA test was performed to assess if there was any statistically significant difference in  $\Delta Q$ -Baseline between the lesions assigned to the seven groups. No statistically significant differences were found.

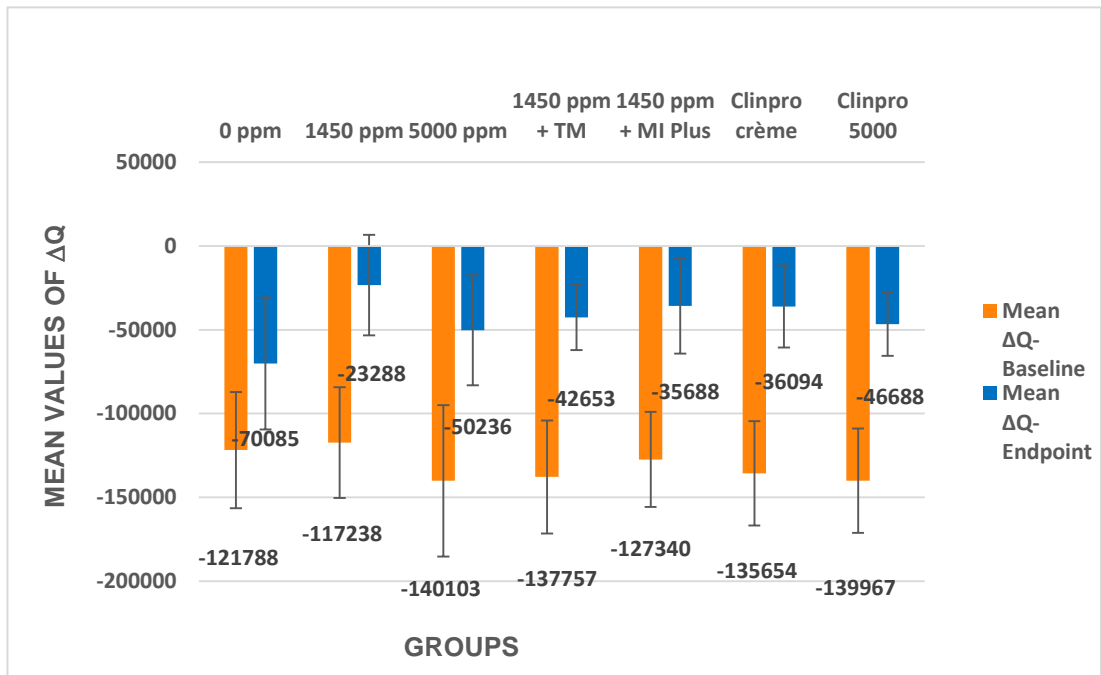
#### **3.2.1. Difference in $\Delta Q$ within each group**

The mean values of  $\Delta Q$ -Baseline and  $\Delta Q$ -Endpoint are shown in Table 6. The table demonstrates an improvement in  $\Delta Q$  values for all the groups in the study.

Figure 20 shows the change in the mean of  $\Delta Q$ -Baseline and  $\Delta Q$ -Endpoint with the standard deviation for all groups. It is clear that there was a reduction in all values of  $\Delta Q$ -Endpoint in all groups.

Groups	Mean ΔQ- Baseline	Mean ΔQ- Endpoint	Mean ΔQ- Difference	95% CI of the Difference		Sig. (2- tailed)
	± SD	± SD	± SD	Lower	Upper	
0 ppm F	-121788 ± 34672	-70085 ± 39317	51703 ± 29532	39775	63631.6	0.000*
1450 ppm F	-117238 ± 33059	-23288 ± 29970	93949 ± 38567	78372.3	109527.4	0.000*
5000 ppm F	-140103 ± 45123	-50236 ± 32817	89867 ± 44867	71749	10798.2	0.000*
1450 ppm F + Tooth Mousse	-137757 ± 33711	-42653 ± 19438	95104 ± 31020	82575.2	107633.7	0.000*
1450 ppm F + MI Paste Plus	-127340 ± 28408	-35688 ± 28438	91651 ± 23964	81972.3	101331.2	0.000*
Clinpro Crème	-135654 ± 31145	-36094 ± 24567	99559 ± 28693	87970.2	111149.2	0.000*
Clinpro 5000	-139967 ± 31134	-46688 ± 18913	93279 ± 25555	82957.2	103601.4	0.000*

**Table 6: Paired sampled T-Test results for ΔQ values at baseline and after treatment**



**Figure 20: The means of both  $\Delta Q$ -Baseline and  $\Delta Q$ -Endpoint values for all groups**

In order to check if the values of  $\Delta Q$ -Endpoint were normally distributed, data normality tests were carried out. This included a Shapiro-Wilk test and a Kolmogorov-Smirnov test. For all groups, except the 0 ppm F toothpaste group, 1450 ppm F toothpaste group, and (1450 ppm F toothpaste + MI Paste Plus) group, the P value was not statistically significant therefore data was considered normally distributed ( $P > 0.05$ ).

To assess whether the change in  $\Delta Q$  at baseline and after treatment was statistically significantly different within the same group, paired T-tests were carried out where the data were normally distributed.

In groups: 0 ppm F toothpaste, 1450 ppm F toothpaste, and (1450 ppm F toothpaste + MI Paste Plus), the data were not normally distributed, therefore the non-parametric Wilcoxon test was used.

The results in Table 6 show that there was a statistically significant improvement in the  $\Delta Q$  values after treatment compared with that at baseline in all groups ( $P < 0.05$ ).

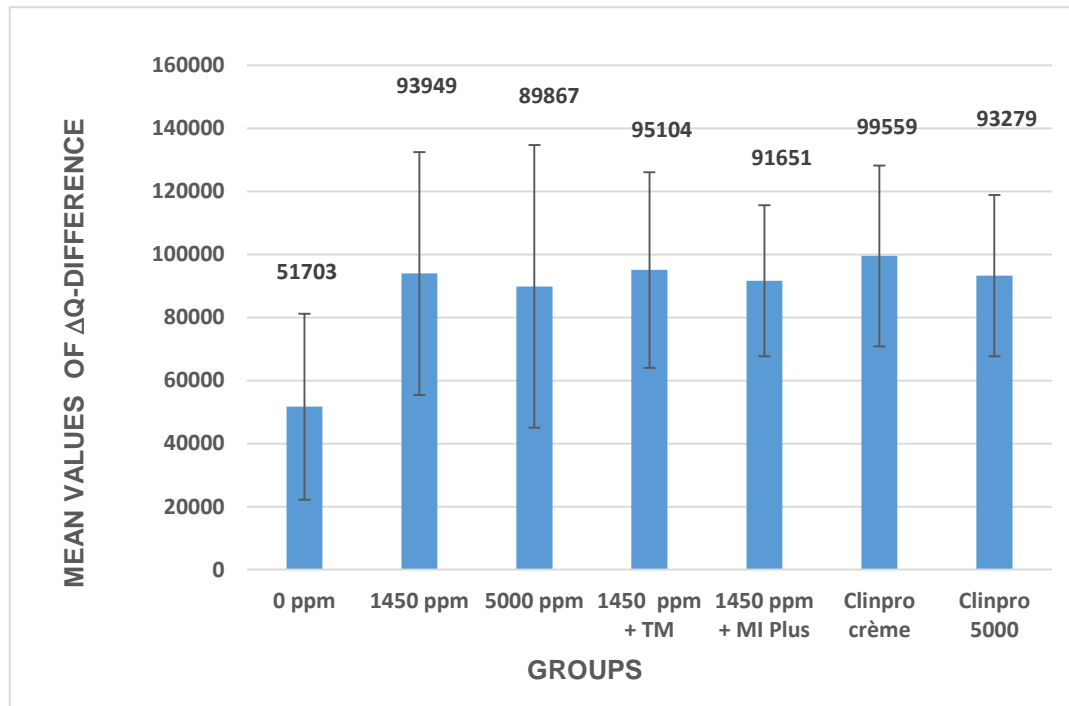
### 3.2.2. Difference in $\Delta Q$ between groups

The  $\Delta Q$ -Difference (change) was measured using the following formula:

$$\text{Difference in } \Delta Q = \Delta Q \text{ after treatment} - \Delta Q \text{ at baseline}$$

In all groups the difference in  $\Delta Q$  was positive, meaning that there was decrease in  $\Delta Q$ -Endpoint compared to  $\Delta Q$ -Baseline. The highest reduction in  $\Delta Q$  was in the Clinpro Tooth Crème group with a mean difference of  $(99559.7 \pm 28693.4)$ , while the lowest reduction was in the 0 ppm F toothpaste group with only  $(51703.3 \pm 29532.2)$  mean difference.

Figure 21 shows the change in the mean of  $\Delta Q$ -Difference with the standard deviation for all groups.



**Figure 21: Means of the  $\Delta Q$ -Difference of all groups**

Normality tests including the Shapiro-Wilk test and Kolmogorov-Smirnov test were carried out to check if the  $\Delta Q$ -Difference for inter-group comparisons were normally distributed. The data were considered normally distributed, as there were no statistically significant differences.

A one way ANOVA test (Table 7) was performed to assess if the  $\Delta Q$ -Difference was statistically significant between the seven groups. It showed that the mean of  $\Delta Q$ -Difference was statistically significant between the groups ( $P < 0.05$ ).

	Sum of Squares	df	Mean Square	F	Sig.
<b>Between Groups</b>	<b>9985521110.613</b>	<b>6</b>	<b>6853314932</b>	<b>6.496</b>	<b>.000*</b>
<b>Within Groups</b>	<b>176627966444.833</b>	<b>175</b>	<b>1054949236</b>		
<b>Total</b>	<b>186613487555.446</b>	<b>181</b>			

**Table 7: One-way ANOVA between groups for the  $\Delta Q$ -Difference**

In order to determine which groups were statistically significant different, pairwise comparison was performed (Table 8). The test showed that 0 ppm F toothpaste had a significantly lower reduction in  $\Delta Q$ -Difference when compared to all other groups ( $P < 0.05$ ).

	Mean Difference	Std. Error	Sig.	95% Confidence Interval	
				Lower	Upper
0 ppm F - 1450 ppm F	-42246.60	9008.32	.000*	-70019.95	-14473.26
0 ppm F - 5000 ppm F	-38163.79	9008.32	.001*	-65937.14	-10390.45
0 ppm F - (1450 ppm F + Tooth Mousse)	-43401.19	9008.32	.000*	-71174.53	-15627.84
0 ppm F - (1450 ppm F + MI Paste Plus)	-39948.47	9008.32	.000*	-67721.82	-12175.13
0 ppm F- Clinpro Tooth Crème	-47856.42	9008.32	.000*	-75629.77	-20083.07
0 ppm F - Clinpro 5000	-41576.05	9008.32	.000*	-69349.39	-13802.70
1450 ppm F - 5000 ppm F	4082.80	9008.32	1.000	-23690.53	31856.15
1450 ppm F - (1450 ppm F + Tooth Mousse)	-1154.58	9008.32	1.000	-28927.93	26618.76
1450 ppm F - (1450 ppm F + MI Paste Plus)	2298.12	9008.32	1.000	-25475.21	30071.47
1450 ppm F - Clinpro Tooth Crème	-5609.81	9008.32	1.000	-33383.16	22163.52
1450 ppm F - Clinpro 5000	670.55	9008.32	1.000	-27102.79	28443.90
5000 ppm F - (1450 ppm F + Tooth Mousse)	-5237.39	9008.32	1.000	-33010.74	22535.95
5000 ppm F- (1450 ppm F + MI Paste Plus)	-1784.68	9008.32	1.000	-29558.02	25988.66
5000 ppm F- Clinpro Tooth Crème	-9692.62	9008.32	1.000	-37465.97	18080.71
5000 ppm F- Clinpro 5000	-3412.25	9008.32	1.000	-31185.59	24361.09
(1450 ppm F + TM) - (1450 ppm F + MI Paste Plus)	3452.71	9008.32	1.000	-24320.63	31226.05
(1450 ppm F + Tooth Mousse) - Clinpro Tooth Crème	-4455.23	9008.32	1.000	-32228.58	23318.11
(1450 ppm F + Tooth Mousse) - Clinpro 5000	1825.14	9008.32	1.000	-25948.20	29598.48
(1450 ppm F + MI Paste Plus) - Clinpro Tooth Crème	-7907.94	9008.32	1.000	-35681.29	19865.40
(1450 ppm F + MI Paste Plus) - Clinpro 5000	-1627.57	9008.32	1.000	-29400.91	26145.77
Clinpro Tooth Crème - Clinpro 5000	6280.37	9008.32	1.000	-21492.97	34053.72

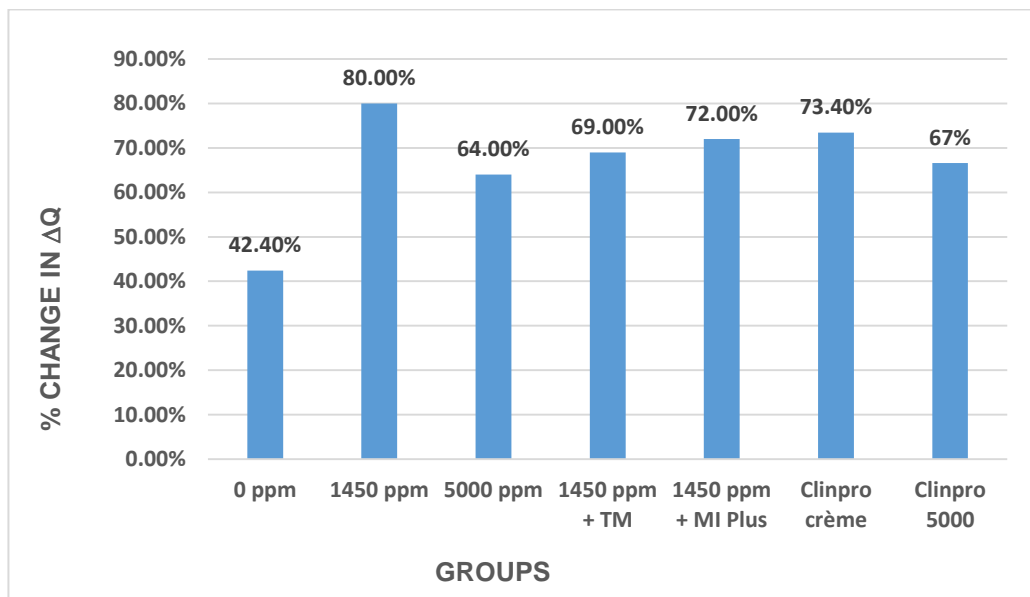
**Table 8: Multiple comparisons of  $\Delta$ Q-Difference between all test groups**

### 3.2.3. The percentage changes in $\Delta Q$

The percentage of the changes in  $\Delta Q$  at baseline and after treatment (% Q) was calculated using the following formula:

$$(\Delta Q\text{-Difference} / \Delta Q\text{-Baseline}) \times 100$$

Figure 22 shows the %  $\Delta Q$  change values for all groups with 1450 ppm F toothpaste group having the highest reduction in  $\Delta Q$  among the groups at 80%, while 0 ppm F toothpaste group shows the lowest reduction percentage at 42.4 %.



**Figure 22: The % change in  $\Delta Q$  for all groups**

### 3.2.4. Intra-examiner reproducibility for $\Delta Q$

The intra-examiner reproducibility was tested using intra-class correlation coefficient. A random selection of 28 enamel slabs which accounts for 15% of the enamel slabs was carried out. These 28 enamel slabs were re-



analysed. The Intra-class Correlation Coefficient was found to be (0.99) which represents excellent reproducibility.

### 3.3. Area of the white spot lesions

The values of the white spot lesion areas at baseline for all groups were checked to see if there was a difference between the groups. The normality tests (Shapiro-Wilk test and Kolmogorov-Smirnov test) showed that the data were normally distributed.

One way ANOVA test (Table 9) was performed to assess if there was any statistically significant differences in the values of Area-Baseline between the lesions assigned to the seven groups. No statistically significant differences were found.

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	6469429.7	6	1078238.3	1.025	.410
Within Groups	184046131.8	175	1051692.2		
Total	190515561.5	181			

**Table 9: One-way ANOVA between groups for the values of Area-Baseline for all groups**

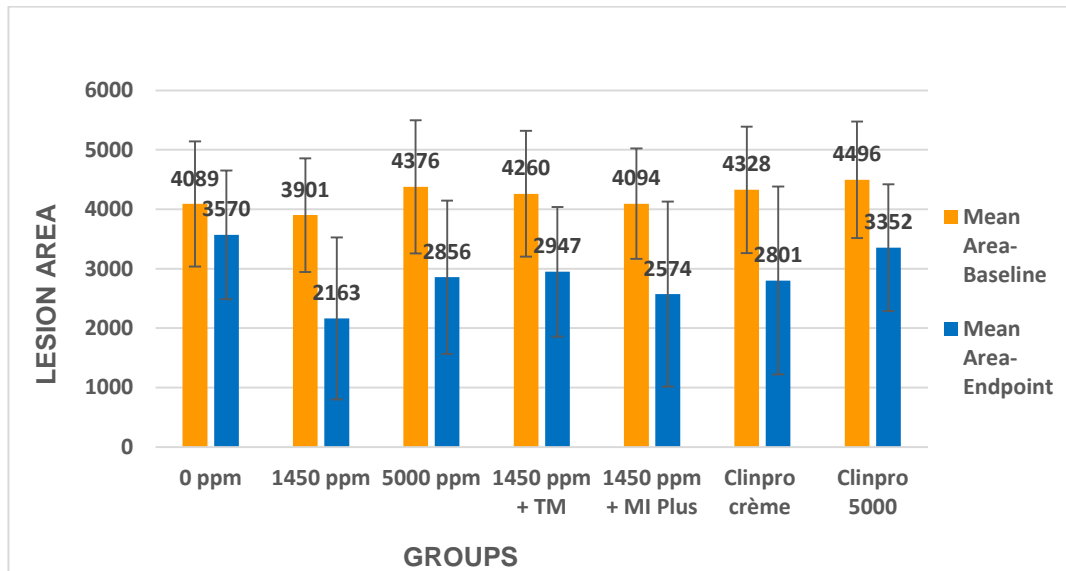
#### 3.3.1. Difference in lesion area within each group

The lesion area mean values both at baseline and after treatment are shown in (Table 10). It is obvious that there was a reduction in the lesion area for all groups in the study.

	Mean Area- Baselin e ± SD	Mean Area- Endpoint ± SD	Mean Area- Differen ce ± SD	95% Confidence Interval of the Difference		Sig. (2- tailed)
				Lower	Upper	
<b>0 ppm F</b>	<b>4089</b> ± <b>1055</b>	<b>3570</b> ± <b>1084</b>	<b>-519</b> ± <b>828</b>	<b>-853.5</b>	<b>-184.5</b>	<b>0.004*</b>
<b>1450 ppm F</b>	<b>3901</b> ± <b>956</b>	<b>2163</b> ± <b>1361</b>	<b>-1737</b> ± <b>1318</b>	<b>-2270</b>	<b>-1205.4</b>	<b>0.000*</b>
<b>5000 ppm F</b>	<b>4376</b> ± <b>1121</b>	<b>2856</b> ± <b>1288</b>	<b>-1519</b> ± <b>1506</b>	<b>-2128.4</b>	<b>-911.5</b>	<b>0.000*</b>
<b>1450 ppm F + Tooth Mousse</b>	<b>4260</b> ± <b>1059</b>	<b>2947</b> ± <b>1090</b>	<b>-1313</b> ± <b>937</b>	<b>-1691.9</b>	<b>-934.3</b>	<b>0.000*</b>
<b>1450 ppm F + MI Paste Plus</b>	<b>4094</b> ± <b>927</b>	<b>2574</b> ± <b>1556</b>	<b>-1520</b> ± <b>1231</b>	<b>-2018.2</b>	<b>-1023.3</b>	<b>0.000*</b>
<b>Clinpro Crème</b>	<b>4328</b> ± <b>1064</b>	<b>2801</b> ± <b>1582</b>	<b>-1527</b> ± <b>1257</b>	<b>-2035.3</b>	<b>-1019.4</b>	<b>0.000*</b>
<b>Clinpro 5000</b>	<b>4496</b> ± <b>979</b>	<b>3352</b> ± <b>1066</b>	<b>-1144</b> ± <b>867</b>	<b>-1494.4</b>	<b>-793.7</b>	<b>0.000*</b>

**Table 10: Paired sampled T test results for the lesion area values at baseline and after treatment for all groups**

Figure 23 shows the change in the mean of Area-Baseline and Area-Endpoint with the standard deviation for all groups.



**Figure 23: Lesion Area at baseline and after treatment for all groups**

To assess whether the change in the area at baseline and after treatment was significantly different within the same group, paired T-Tests were used.

The paired T-Test results shown in (Table 10) showed that there was a statistically significant improvement in the lesion area values after treatment in all groups ( $P < 0.05$ ).

### 3.3.2. Difference in lesion area between groups

The lesion area difference (change) was measured using the following formula:

$$\text{Difference in lesion area} = \text{lesion area after treatment} - \text{lesion area at baseline}$$

Figure 24 shows the Area-Differences for the seven tested groups. In all test groups, the mean Area-Difference was negative indicating a decrease in the area of the lesion after treatment compared to that at baseline.

The greatest reduction in lesion size was observed in 1450 ppm F toothpaste group, whereas the lowest reduction in lesion size was in the 0 ppm F toothpaste group.

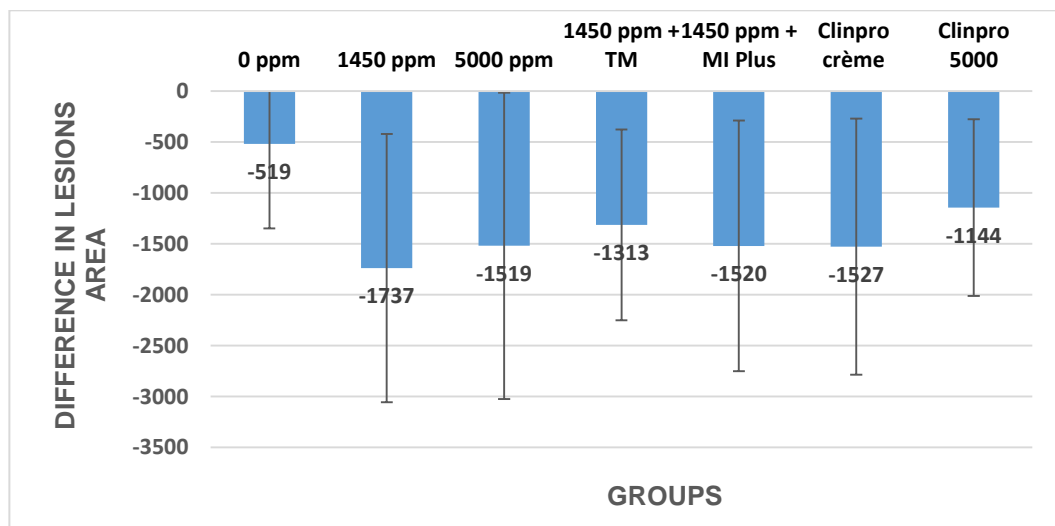


Figure 24: Means of the lesion Area-Difference of all tested groups

In order to check if the lesion Area-Differences were normally distributed, data normality tests were carried out. This included a Shapiro-Wilk test and a Kolmogorov-Smirnov test. The data was not considered normally distributed as there were statistically significant differences.

A non-parametric Kruskal-Wallis test was performed to assess if Area-Difference was statistically significant between the seven groups. The mean Area-Difference was found to be statistically significant between groups ( $P < 0.05$ ).

In order to determine which groups were statistically significantly different, pairwise comparisons were performed (Table 11). The 0 ppm F toothpaste group had a statistically significantly lower mean reduction in area when compared to all groups ( $P < 0.05$ ).

	Test statistic	Std. Error	Std. Test Statistic	Sig.	Adj. Sig
1450 ppm F- Clinpro Tooth Crème	-10.38	14.61	0.711	0.477	1.000
1450 ppm F - (1450 ppm F + MI Paste Plus)	-11.23	14.61	0.79	0.442	1.000
1450 ppm F – (1450 ppm F + Tooth Mousse)	-14.53	14.61	0.99	0.320	1.000
1450 ppm F – 5000 ppm F	-16.15	14.61	1.10	0.269	1.000
1450 ppm F- Clinpro 5000	-18.26	14.61	1.25	0.211	1.000
1450 ppm F- 0 ppm F	63.76	14.61	4.36	0.000	0.000
Clinpro Tooth Crème - (1450 ppm F + MI Paste Plus)	0.84	14.61	0.05	0.954	1.000
Clinpro Tooth Crème - (1450 ppm F + Tooth Mousse)	4.15	14.61	0.28	0.776	1.000
Clinpro Tooth Crème - 5000 ppm F	5.76	14.61	0.39	0.693	1.000
Clinpro Tooth Crème – Clinpro 5000	-7.88	14.61	-0.54	0.589	1.000
Clinpro Tooth Crème – 0 ppm F	53.38	14.61	3.65	0.000	0.005
(1450 ppm F + MI Paste Plus) - (1450 ppm F + Tooth Mousse)	3.30	14.61	0.22	0.821	1.000
(1450 ppm F + MI Paste Plus) – 5000 ppm F	4.92	14.61	0.33	0.736	1.000
(1450 ppm F + MI Paste Plus) - Clinpro 5000	-7.03	14.61	0.48	0.630	1.000
(1450 ppm F + MI Paste Plus) – 0 ppm F	52.53	14.61	3.59	0.000	0.007
(1450 ppm F + Tooth Mousse) – 5000 ppm F	1.61	14.61	0.11	0.912	1.000
(1450 ppm F + Tooth Mousse) – Clinpro 5000	-3.73	14.61	-0.25	0.798	1.000
(1450 ppm F + Tooth Mousse) – 0 ppm F	49.23	14.61	3.36	0.001	0.016
5000 ppm F – Clinpro 5000	-2.11	14.61	0.14	0.885	1.000
5000 ppm F – 0 ppm F	47.61	14.61	3.25	0.001	0.024
Clinpro 5000 – 0 ppm F	45.50	14.61	3.11	0.002	0.039

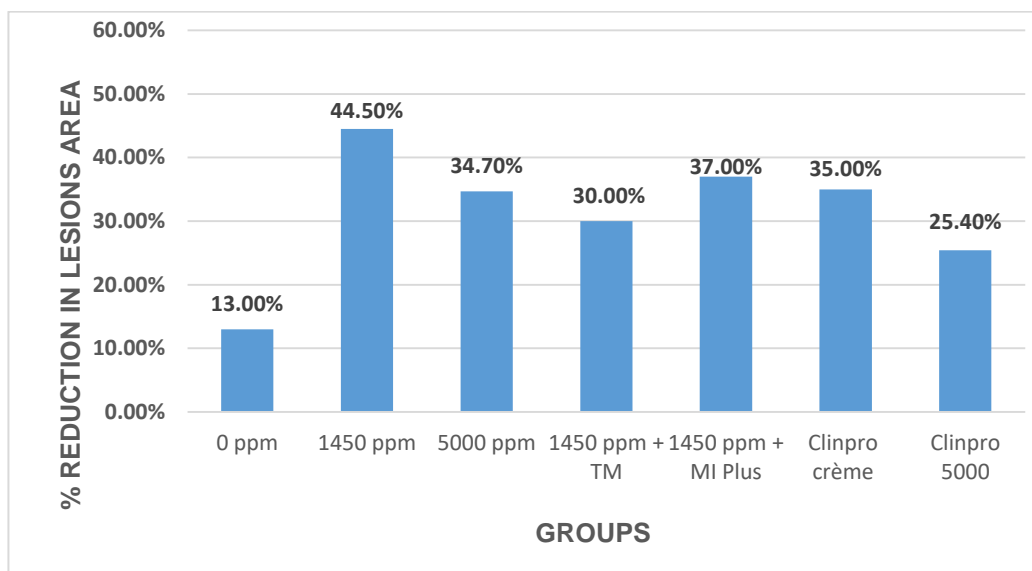
Table 11: Multiple comparisons of Area-Differences between all groups with Bonferroni correction

### 3.3.3. The percentage change in lesion area

The percentage change in lesion area at baseline and after treatment (% Area) was calculated using the following formula:

$$(\text{Area-Difference} / \text{area at baseline}) \times 100$$

Figure 25 shows the % Area values for all groups, with 1450 ppm F toothpaste group having the highest % reduction in lesion area: 44.5%, followed by the 1450 ppm F toothpaste + MI Paste Plus group, Clinpro Tooth Crème group, 5000 ppm F toothpaste, the 1450 ppm F toothpaste+ Tooth Mousse group, Clinpro 5000 group at 37%, 35%, 34.7%, 30%, and 25.4% respectively. The lowest % reduction in area was found in the 0 ppm F toothpaste group at 13%.



**Figure 25: The % reduction in Area values for all groups**

#### **3.3.4. Intra-examiner reproducibility for area of the white spot lesion**

The intra-examiner reproducibility was tested using the intra-class correlation coefficient. A random selection of 28 enamel slabs which accounts for 15% of the enamel slabs was carried out. These 28 enamel slabs were re-analysed. The Intra-class Correlation Coefficient was found to be (0.99) which represents excellent reproducibility.



### 3.4. Summary of the results for all three parameters

#### **The $\Delta F$ results:**

Comparison of  $\Delta F$  values within each group revealed a statistically significant improvement in  $\Delta F$  values after the treatment as compared with baseline in all groups. The improvement in  $\Delta F$  was highest in the Clinpro Tooth Crème group, followed by 1450 ppm F toothpaste + MI Paste Plus group, 1450 ppm F toothpaste group, 1450 ppm F toothpaste + Tooth Mousse group, Clinpro 5000 group, and 5000 ppm F toothpaste groups.

Comparison between the seven different groups showed that the mean difference in  $\Delta F$  of the negative control was significantly lower than the mean difference in  $\Delta F$  of all other test groups except for the 5000 ppm F toothpaste group and the Clinpro 5000 group. Also, the mean of

“ $\Delta F$ -Difference of the Clinpro Tooth Crème” group was significantly higher than the mean of “ $\Delta F$ -Difference of 5000 ppm F toothpaste” group. Different concentrations of NaF toothpastes used in the study did not achieve any significant differences in effect between the groups.

#### **The $\Delta Q$ results:**

Comparison of  $\Delta Q$  values within each group revealed statistically significant improvement of  $\Delta Q$  values after the treatments as compared with before the treatment in all groups. The highest improvement was achieved by the Clinpro Tooth Crème group followed by 1450 ppm F toothpaste + Tooth

Mousse group, 1450 ppm F toothpaste group, Clinpro 5000 group, 1450 ppm F toothpaste + MI Paste Plus group, 5000 ppm F toothpaste group.

Comparison between the seven different groups showed that the improvement in  $\Delta Q$  in the negative control was significantly lower than that for all test groups. No other statistical significance was found between groups.

**The lesion Area results:**

Comparison of Area of the lesion values within each group revealed statistically significant improvement in the lesion Area after the treatments as compared with before the treatment in all groups

Comparison between the seven different groups showed that the improvement in lesion Area in the negative control was statistically significantly lower than that for all test groups. No other statistical significance was found between groups.

## Chapter 4

### DISCUSSION

#### 4.1. *In vitro* models

The present study used an *in vitro* model to investigate the effect of different remineralising agents to remineralise artificial early enamel subsurface lesions in bovine teeth. *In vitro* models are considered to be the most commonly used models in cariology research as they are laboratory-based models in which the conditions associated with the caries process can be simulated in a well-controlled environment. This reduces the variability that is inherent in both *in situ* studies and clinical trials (White, 1995).

*In vitro* models in general are easy to conduct and requires less time and smaller sample sizes as compared with *in situ* studies and clinical trials (Zero, 1995). This model when used in cariology research, is able to provide repeatability and reproducibility between tests which is an important validation tool in research field (Buzalaf et al., 2010).

However, there are some limitations that are inherent in *in vitro* models which should be kept in mind particularly before applying the data which is derived from such models in clinical situations. For example, it is difficult to completely replicate the complex biological and physiological intra-oral conditions associated with the caries process; such as the oral biofilm, salivary flow, dental pellicle, and interaction between them. Also this model is unable to mimic natural clearance of products from the oral cavity (White, 1992).

## 4.2. Study design

The present study investigated the effect of seven products in remineralising artificial early enamel subsurface lesions and compared them with the effect of 0 ppm F toothpaste (negative control) and 1450 ppm F toothpaste as a positive control. The test products were used according to manufacturer recommendations and included: Tooth Mousse, MI Paste Plus, Clinpro Tooth Crème, Clinpro 5000 and high fluoride toothpaste (5000 ppm F). In order to prevent any bias in the study, the study design was randomised, single-blinded.

## 4.3. Bovine teeth

Although human teeth are preferred in cariology research, in the current study bovine teeth were used instead. This because sound human teeth are difficult to collect in large numbers. In fact, bovine teeth are used more often than human teeth in cariology research due to a number of advantages listed below:

- The ease of obtaining bovine teeth in large quantity and in good condition (Melberg, 1992).
- Bovine teeth have wider and larger surface area as compared to human teeth, which means more enamel slabs can be obtained from a single bovine tooth (Edmunds et al., 1988).

- Artificial caries lesions created in the bovine teeth have similar mineral composition and structure to caries lesions in human teeth (Featherstone et al., 1981).
- Bovine teeth have more porous enamel than human teeth. This means that the remineralisation and demineralisation process is faster in bovine teeth than in human teeth. This means that less experimental time is required to show the effect to be studied (Lynch and ten Cate, 2006; Featherstone et al., 1981).

Due to these advantages, bovine teeth were used instead of human teeth.

An important aspect that should be considered prior to the use of bovine teeth is the occurrence of an infectious disease called bovine spongiform encephalopathy (BSE), or mad cow disease. This disease attacks bovines and produces a progressive neurodegenerative lesion, by means of an infectious prion [Prusiner, 1982]. In order to eliminate this infectious disease teeth were immersed in 12% sodium hypochlorite for 24 hours to eliminate prions. A previous study showed that sodium hypochlorite did not have an effect on the mineral content of dentine or its crystal structure (Driscoll et al., 2002).

#### **4.4. Enamel slabs preparation and storage**

In the present study, the buccal section of the sectioned bovine tooth was used because it is thicker and has more flat surface area as compared with the lingual surface.

Once enamel slabs were created, they were stored in 0.1% thymol to maintain them and preserve 100% humidity. The 0.1% thymol is the most commonly used storage solution. Thymol solution is known to prevent bacterial growth due to its antibacterial properties (Titley et al., 1998).

Research have shown that storage of teeth in 0.1% thymol has a minimal effect on enamel structure with no significant effect on enamel strength and permeability (Secilmis et al., 2013; Tosun et al., 2007; Ziskind et al., 2003).

#### **4.5. Artificial caries lesions**

In cariology studies, artificial caries lesions are initially produced by immersion of the slabs in a solution with a low pH ranging between 4.4- 5.0 for a time ranging between 16 hours to 28 days. Two solutions can be used: buffered solution or acidified gel which would influence the type of the lesions created (White, 1987).

Depending on the protocol used, two types of lesions can be created: erosion-like lesions with softened surface, or caries-like lesions also known as subsurface lesions. In the current study, caries-like lesions were produced to test the study's products ability to remineralise lesions (Lynch et al., 2007). Thus, acidified hydroxyethyl cellulose gel was used to create sub-surface caries-like lesions. This gel contains calcium, phosphate, and fluoride in order to prevent the etching of the enamel surface and promote subsurface enamel dissolution simulating natural caries progression (ten Cate and Duijsters, 1983).

The enamel slabs were immersed in the acidified gel for 10 days in order to produce enamel lesions with  $\Delta F$ s of similar range.

## 4.6. pH cycling

The model used in this study was a pH cycling *in vitro* model in which enamel slabs were subjected to combinations of demineralisation and remineralisation solutions with daily treatments with the test products. This model is broadly used in cariology studies because it simulates the real alteration of oral pH during bacterial sugar metabolism and caries formation. It is designed to simulate the dynamics of mineral loss and gain involved in the caries process, which is an important advantage of the pH-cycling model. This advantage enables researchers to study the caries process and understand the mechanism of action of test products such as fluoride dentifrices (White, 1995). This model is also characterised by a high level of scientific control that would reduce the variability intrinsic to *in vitro* models. Other advantages of this model are simplicity, low cost, efficiency in time saving, reproducibility, and stability (Skucha-Nowak et al., 2015). Additionally, this model requires smaller sample sizes as compared to other models (Buzalaf et al, 2010). The information derived from such models can be used to help designing clinical trials (Zero, 1995, Roberts, 1995). However, pH-cycling models as all *in vitro* models have important limitations as follows (White, 1995; 1992):

- Inability to completely simulate the complex intraoral conditions associated with caries process.
- Inability to mimic solid surface/solution ratios or saliva/plaque fluid composition.

- The time periods of demineralisation and remineralisation are faster than those expected to occur *in vivo* conditions.
- Inability to simulate topical use and natural clearance of products from the oral cavity.

The genesis of current pH-cycling models was produced by ten Cate and Duijsters (1982).

The pH cycling protocol used for the current study was developed at the University of Leeds and has been used in previous caries studies at the University of Leeds-Paediatric Dentistry Department.

#### **4.7. Quantitative Light-induced Fluorescence**

In the present study, quantitative light-induced fluorescence has been used to detect and quantify changes in enamel mineral content and size of lesions.

This non-invasive method aids in detecting early caries lesions and monitoring progression or regression over the time (Pretty, 2006).

It is able to detect and quantify the contrast in fluorescence between sound enamel and demineralised enamel. The quantification process enables researchers to detect any minor changes in the mineral status within the caries lesion, hence reducing the duration of the study (Pretty, 2006).

This method has been rapidly adopted as a standard measure in studying the effectiveness of preventive treatments and it has been shown to be a sensitive, valid, and reproducible detection tool for caries lesions (Karlsson, 2010, Feng et al., 2007, Stookey, 2004).



Previous studies, validating Quantitative Light-induced Fluorescence with the currently accepted gold standard for the measurement of mineral loss in natural and artificial caries lesions; Transverse Micro-Radiography, found a strong correlation between the two techniques in detecting early mineral loss in caries process (Lagerweij et al., 1999; Alkhatteb et al., 1997; Ando et al., 1997).

Quantitative Light-induced Fluorescence has shown high sensitivity and specificity in detecting enamel lesions (Angmar-Mansson and Bosch, 2001; Shi et al., 2001; Josselin et al., 1995).

In the current study the intra-class correlation coefficients (ICCs) for intra-examiner reliability of the image analysis was found to be 0.99 which represents a high reproducibility. This was in line with previous studies which showed high reproducibility of the analysis stage of the QLF technique, both in terms of intra- and inter-examiner agreement of QLF (Gomez et al., 2013; Pretty et al., 2002).

However, many confounders can influence the QLF analysis outcome including the skills of the operator, presence of plaque or calculus, degree of moisture, shadowing, and the presence of enamel defects (Pretty et al., 2002).

Thus in order to avoid these negative influences the following were performed:

- Any tooth with enamel defects or staining was excluded from the study.
- All images were taken under standardised and controlled conditions.
- All slabs were dried for 15 seconds with compressed air prior to QLF imaging.

- All QLF Images were captured under darkroom conditions.
- The camera was fixed in the same position on a stand during QLF image capture.
- Training for using QLF device and software were obtained prior to the study in order to reduce any operator bias.
- Repeated QLF analysis was performed to reduce measurement errors.

Some reports suggest that QLF may be limited by lesion depth. When compared to electrical conductance, QLF method was more sensitive in measuring shallow occlusal lesions. However, QLF was deficient in discriminating deeper lesions. This is because of the limited penetration of light into enamel in QLF method. The light source can provide light with a wavelength that makes QLF effective in quantifying enamel lesions up to 400  $\mu\text{m}$  in depth, but not beyond (Al-Khateeb et al., 1997)

Another limitation of QLF method is the subjectivity of analysis stage of the stored tooth image which increases the potential for operator bias. The analysis of QLF images includes determination of the demineralised enamel and its borders and the placement of the analysis patch. These subjectivities of QLF method can have an effect on the reported  $\Delta F$  value (Pretty et al., 2002).

#### **4.8. Results of Quantitative Light-induced Fluorescence**

In this laboratory study, three QLF parameters were calculated; the  $\Delta F$  which represents the percentage fluorescence loss and is related to lesion depth, the surface area of the lesion and the  $\Delta Q$  which is the  $\Delta F$  times the area and represents the lesion volume. Ando et al., 2004 has shown in his study that both  $\Delta F$  and size of lesion have variable correlation. He showed that the  $\Delta F$  value may be maintained even if the size of the lesion increases or decreases, or the size of the lesion may be maintained, although the  $\Delta F$  value may increase or decrease. He concluded that both parameters were not accurate indicators of the severity of the caries lesion. Therefore, the  $\Delta Q$  value should be considered as the main indicator for net remineralisation (Ando et al., 2004).

#### **4.9. Remineralising potential of fluoridated toothpastes on artificial early enamel subsurface lesions**

The present study investigated the effect of fluoridated toothpastes on remineralising artificial early enamel subsurface lesions. Two fluoridated toothpastes with different NaF concentrations were assessed; the 1450 ppm F toothpaste, and the 5000 ppm F toothpaste, along with 0 ppm F toothpaste as a negative control.

At the end of the study, remineralisation of the early enamel subsurface lesions were found in all the three groups. The highest remineralisation was produced by the 1450 ppm F toothpaste followed by that produced by 5000 ppm F toothpaste and the least remineralisation was produced in the negative control group, 0 ppm F toothpaste.

When  $\Delta Q$  and lesion Area values were compared, both the 1450 ppm F toothpaste and the 5000 ppm F toothpaste were significantly more effective than the 0 ppm F toothpaste (negative control) in remineralising artificial early enamel subsurface lesions. The comparison of the  $\Delta F$  values between the groups showed a statistically significant difference between the 1450 ppm F toothpaste and the 0 ppm F toothpaste, but no statistically significant difference between the 5000 ppm F toothpaste and the 0 ppm F toothpaste. No statistically significant difference was found between the 1450 ppm F toothpaste and the 5000 ppm F toothpaste for any of the three parameters.

Thus the results showed that the 1450 ppm F toothpaste yielded more remineralisation than the 5000 ppm F toothpaste, although the difference did not reach statistical significance. Moreover, no statistically significant difference was found between the 5000 F toothpaste and the 0 ppm F toothpaste (negative control) when  $\Delta F$  values were compared. This was an unexpected finding, knowing that a positive relationship between fluoride dose and effect on remineralisation has been reported in the literature.

Marinho et al., in a Cochrane report that was conducted in 2003, reported that the remineralising effect of fluoride toothpastes appears to increase with higher fluoride concentrations in the toothpaste. High fluoride toothpaste

(5000 ppm F) significantly reduced the caries progression in adolescents aged 14–16 years when compared with 1450 ppm F toothpaste over two years in a randomised controlled clinical trial (Nordstrom and Birkhed, 2010).

This was in line with the result of another clinical trial conducted by Sonesson et al. (2014) on orthodontic patients. The trial found that 5,000 ppm F toothpaste resulted in a statistically lower white spot lesion incidence (18.1%) compared with the 1,450 ppm F toothpaste (26.6%). Also, using a pH-cycling model, ten Cate in his *in vitro* study in 2008 was able to demonstrate that a higher fluoride toothpaste (5000 ppm F) resulted in more remineralisation when compared with traditional fluoride products (1500 ppm F) in advanced subsurface lesions (ten Cate et al., 2008)

A possible explanation for why the result of the current study did not follow the expected dose effect response is that the high concentration of fluoride (5000 ppm F) could have precipitated as calcium fluoride on the surface of the lesions leading to blocking of the surface layer pores and reduction in the permeability of the enamel and this would inhibit the subsurface area remineralisation and subsequently affect the depth of the lesions. Research reported that calcium fluoride was a major product on enamel when teeth were exposed to high concentrations of fluoride (Gerould, 1945). Some evidence to support this hypothesis can be found in the literature. ten Cate et al. (1981) found that the rate of mineral deposition was higher initially, but lower subsequently when high-concentration fluoride solutions were applied topically. They postulated that fluoride could have resulted in blocking of the surface layer pores.

This inhibitory effect of fluoride was also observed when comparison between fluoride-free solution and 1-2 ppm fluoride solution in remineralisation of the body of the caries lesions *in vitro* was performed (Lammers et al., 1990; Kawasak, 1989)

Moreover, some studies in the literature also showed that a complete remineralisation of the caries lesion cannot be achieved when the surface layer has been fully remineralised with high concentration of fluoride (Greene and Newbrun, 1986. Dirks, 1966)

This precipitation of fluoride in the surface layer can block the enamel pores and subsequently prevent the diffusion of minerals deep into the lesions. This will produce an enamel surface that is much more caries-resistant compared to the original enamel with the demineralised subsurface area still present underneath (ten Cate and Duijsters, 1982). It could be argued that this is not always a problem if caries process can be stopped with retained demineralised subsurface lesion. This could be accepted for noncompliant patients with high caries risk. However, for highly motivated and compliant patients with caries lesions at early stages any intervention should be aimed to enhance remineralisation.

#### **4.10. Remineralising potential of Calcium Phosphate delivering technologies on artificial early enamel subsurface lesions**

In the current study the effect of Clinpro Tooth Crème and Clinpro 5000 on the remineralisation of artificial early enamel subsurface lesions were investigated. Both products were used according to the manufacturer's recommendation.

The results showed that in all QLF parameters ( $\Delta F$ ,  $\Delta Q$  and lesion area) Clinpro Tooth Crème and Clinpro 5000 produced remineralisation of the enamel subsurface lesions which was statistically significant compared with the baseline.

The net remineralisation produced by these products, which is expressed by  $\Delta Q$ , was not found to be different than that produced by the 1450 ppm F and 5000 ppm F toothpastes as it failed to reach significant levels. This might be attributed to the limitation of the *in vitro* model, which might not be sensitive enough to express these differences. Moreover, the duration of the study might not be long enough to express any difference in the effectiveness of the test products. Research reported that CPP-ACP was successful in remineralising subsurface enamel lesions and the extent of remineralisation achieved was dose dependent and increased with increasing the time of exposure and the duration of the study. Hegde et al in 2007 investigated the remineralising potential of CPP-ACP when applied twice a day for five different durations of treatment; 7, 14, 21, 28, and 35 days. The results

showed that the greatest remineralisation of enamel lesions was observed in the samples kept for 35 days.

Difference in the mean values of fluorescence loss in the Clinpro 5000 group was similar to that observed in both the 5000 ppm F toothpaste and the 0 ppm F toothpaste (negative control). A possible explanation of this outcome was discussed earlier.

As far as Clinpro Tooth Crème is concerned, the current study found that the highest remineralisation among all test products was achieved by Clinpro Tooth Crème, however it did not reach statistical significance with respect to the other products.

Therefore, the outcomes of the present study has indicated that the remineralising effectiveness of Tri-Calcium Phosphate technologies did not differ statistically than 1450 ppm F and 5000 ppm F toothpaste.

It should be noted that Clinpro Tooth Crème which contains 950 ppm F has less fluoride concentration than 1450 ppm F and 5000 ppm F toothpastes. Fluoride concentration of dentifrices is one of the main elements of their effectiveness and research has shown that every 500 ppm increase in fluoride concentration in dentifrices produces 6% increase in their effectiveness (Pessan et al., 2011).

The present study indicated that Clinpro Tooth Crème can be a promising agent for remineralising early enamel subsurface lesions but more studies are needed to extract conclusive evidence.

The current study also evaluated the effect of CPP-ACP cream with and without fluoride when associated with regular fluoride dentifrice on demineralised enamel using QLF in a pH-cycling model, simulating oral



cavity conditions. Both pastes were applied once daily after the use of 1450 ppm F toothpaste according to the manufacturer's recommendation.

The results showed that in all QLF parameters ( $\Delta F$ ,  $\Delta Q$  and lesion area) the (1450 ppm F toothpaste + Tooth Mousse) and (1450 ppm F toothpaste + MI Paste Plus) produced significantly more remineralisation of the enamel subsurface lesions as compared with the baseline as well as in comparison to the remineralisation produced in the negative control group; 0 ppm Fluoride toothpaste.

The net remineralisation of these products, which is reflected by  $\Delta Q$ , was not found to be different compared with that produced by the 1450 ppm F and 5000 ppm F toothpastes as it failed to reach a significant level. This might be attributed to the limitation of the *in vitro* model, which might not be sensitive enough to express these differences. Also another factors that may affect the results are the duration of the study and the protocol used.

Conflicting results are found in the literature about the superiority of Tooth Mouse and MI Paste Plus over fluoridated toothpastes. Shen et al. (2011) in his *in situ* trial compared 1000 ppm F, 5000 ppm F, Tooth Mousse, MI Paste Plus, and Clinpro Tooth Crème and was able to show that Tooth Mousse and MI Paste Plus achieved significantly more remineralisation of early enamel subsurface lesions than 5000 ppm F toothpaste. It was found that Tooth Mousse achieved 24% remineralisation of enamel as compared to 29% by MI Paste Plus and only 16% by 5000 ppm F toothpaste.

On the other hand, a more recent *in situ* study that aimed to investigate any additional benefit of the use of Tooth Mousse over the use of fluoridated toothpaste, showed that a 1400 ppm F toothpaste was significantly superior

to the combination of 1400 ppm F toothpaste and Tooth Mousse (Meyer-Lueckel et al., 2015).

The present study failed to demonstrate any significant difference between fluoride products and either Tooth Mousse or MI Paste Plus. This was in line with the result of a randomised clinical trial conducted by Brochner et al. (2011) which failed to demonstrate any additional benefit of using Tooth Mousse over fluoridated toothpastes alone. The assessment of lesions by Quantitative Light-Induced Fluorescence showed that 1100 ppm F toothpaste was as effective as Tooth Mousse in reducing the white spot lesions size after 4 weeks of intervention. Moreover, Li et al. (2014) carried out a systematic review, based on eight studies, and concluded that Tooth Mousse has a long-term remineralisation effect on early caries lesions in comparison with placebos, although this effect was not significantly different from that of other fluoride agents. The review concluded that the clinical benefits of using CPP-ACP supplements over fluoride are still unclear.

In the current study both Tooth Mousse and MI Paste Plus were used directly after the use of the 1450 ppm F toothpaste and this might cause a blockage of the surface pores of the lesions by the formation of fluorapatite in the surface layer which in turn may have prevented the diffusion of the calcium and phosphate ions from the CPP-ACP into the body of the lesion as mentioned earlier. This was confirmed in a recent *in vitro* study which was conducted to explore how the application sequence of CPP-ACP and fluoridated toothpastes influences the remineralisation of enamel white spot lesions in primary teeth. The results of the study indicated that CPP-ACP

followed by fluoridated toothpaste is preferred over fluoridated toothpaste followed by CPP-ACP (Al-Batayneh et al., 2017).

Many studies reported in the literature have proven the superiority of MI Paste Plus over Tooth Mousse in remineralising early enamel subsurface lesions. Oliveira et al. (2014), showed that MI Paste Plus outperformed Tooth Mousse in terms of Area of the lesion observation when both were assessed by QLF in an *in vitro* model.

Another recent *in vitro* study using DIAGNOdent™ was able to show that MI Paste Plus was more effective than Tooth Mousse (Jayarajan et al., 2011). These were in accordance with the results of an *in situ* trial conducted by Shen et al. (2011) which found that that Tooth Mousse achieved 24% remineralisation of enamel as compared to 29% by MI Paste Plus.

In the present study, based on fluorescence loss and Area of lesion measurements, the remineralisation produced by the 1450 ppm F toothpaste + MI Paste Plus group was found to be greater in comparison with that produced by the 1450 ppm F toothpaste + Tooth Mousse group. However, this difference failed to reach a significant level. This was in accordance with the results of a previous study conducted by Bataineh et al. (2017).

Moreover, Mehta et al. (2013) in his *in vitro* study failed to demonstrate any significant difference in the remineralisation that was achieved by either MI Paste Plus or Tooth Mousse. The results showed that Tooth Mousse was as effective as MI Paste Plus in remineralising artificial early enamel subsurface lesions.

It was suggested that CPP-ACP molecules need an acid challenge to be activated to separate ACP from the casein (Reynolds and Walsh, 2005). In this study CPP-ACP was applied after the acid attacks, and acid was washed away before application. This could render these products ineffective and the remineralising effect that was observed could have been produced by the 1450 ppm F toothpaste.

#### 4.11. Suggestions of future research

In the present study Tooth Mousse and MI Paste Plus were used according to the manufacturer's recommendations that is one application after the 1450 ppm F toothpaste. Some evidence in the literature suggested an inhibitory effect of fluoride on the surface of enamel. Therefore, it is worthwhile to consider studying the effect of application sequences of fluoridated toothpastes and CPP-ACP products in remineralising early enamel subsurface lesions.

Moreover, in the present study the application of CPP-ACP pastes was performed after the acid challenges. However, the literature suggests that CPP-ACP products have more preventive effect against caries lesions and acid attacks than a remineralising effect. This is related to an inhibition of demineralisation particularly when CPP-ACP paste is applied before an acid attack (Hicks and Flaitz, 2006). Therefore, it is recommended to investigate the preventive effect on demineralisation of these products in future research. The present study used an *in vitro* model to assess the remineralisation effect of different technologies which is a very useful model to study such effects in the cariology research. However, before translating these results into the clinical situation, well-conducted randomised clinical trials will be required to assess the effectiveness of these technologies described in this study. Other assessment tools rather than QLF should be considered such as Microhardness testing, Scanning Electron Microscopy, or Microcomputed Tomography.

#### 4.12. Null hypotheses

1. The null hypothesis “There is no difference in the enamel remineralisation that results from 1450 ppm F toothpaste + Tooth Mousse, 1450 ppm F toothpaste + MI Paste Plus, Clinpro Tooth Crème, Clinpro 5000, 1450 ppm F toothpaste, and 5000 ppm F toothpaste” can be accepted as no significant differences were found in the enamel remineralisation between calcium phosphate delivering technologies and fluoridated toothpastes.
2. The null hypothesis “There is no difference in the enamel remineralisation that results from CPP-ACP with and without fluoride when used supplementary to 1450 ppm F toothpaste” can be accepted.

## CONCLUSION

From the results of this *in vitro* study it can be concluded that:

- 1- Comparison with the baseline showed a statistically significant remineralisation of artificial early enamel subsurface lesions in all groups.
- 2- All group products were significantly more effective as remineralising agents of artificial early enamel subsurface lesions as compared with the negative control 0 ppm F.
- 3- Calcium phosphate delivering technologies were as effective as fluoridated toothpastes (1450 ppm F and 5000 ppm F) in the remineralisation of artificial early enamel subsurface lesions of bovine teeth assessed using QLF technology.
- 4- Tooth Mousse was as effective as MI Paste Plus when used in conjunction with 1450 ppm F toothpaste in remineralising artificial early enamel subsurface lesions of bovine teeth assessed using QLF technology.

## REFERENCES

- Aimutis, W. 2004. Bioactive properties of milk proteins with particular focus on anticariogenesis. *Journal of Nutrition*. 134(4), pp. 989-995.
- Al-Batayneh, O., Jbarat, R. and Al-Khateeb, S. 2017. Effect of application sequence of fluoride and CPP-ACP on remineralization of white spot lesions in primary teeth: An *in-vitro* study. *Archives of Oral Biology*. 83, pp. 236–240.
- Al-Khateeb, S., ten Cate, J. and Angmar-Mansson, B. 1997. Quantification of formation and remineralization of artificial enamel lesions with a new portable fluorescence device. *Advances in Dental Research*. 11(4), pp. 502-506.
- American Academy of Pediatric Dentistry. 2015. Guideline on fluoride therapy. Reference Manual. 37(6), pp. 15-16.
- Ando, M., Eckert, G., Stookey, G. and Zero, D. 2004. Effect of imaging geometry on evaluating natural white-spot lesions using quantitative light-induced fluorescence. *Caries Research*. 38(1), pp. 39-44.
- Ando, M. van Der Veen, M. Schemehorn, B. Stookey, G. 2001. Comparative study to quantify demineralised enamel in deciduous and permanent teeth using laser and light-induced fluorescence techniques. *Caries Research*. 35, pp. 464-470.
- Ando, M., Hall, A., Eckert, G., Schemehorn, B., Analoui, M. and Stookey, G. 1997. Relative ability of laser fluorescence techniques to quantitate early mineral loss *in vitro*. *Caries Research*. 31(2), pp. 125-131.
- Angmar-Mansson, B. and Ten Bosch, J. 2001. Quantitative light-induced fluorescence (QLF): A method for assessment of incipient caries lesions. *Dentomaxillofacial Radiology*. 30(6), pp. 298–307.



- Azrak, B., Callaway, A., Knozinger, S. and Willershausen, B. 2003. Reduction of the pH-values of whole saliva after the intake of apple juice containing beverages in children and adults. *Journal of Oral Health and Preventive Dentistry*. 1(3), pp. 229-236.
- Bailey, D., Adams, G., Tsao, C., Hyslop, A., Escobar, K., Manton, D., Reynolds, E. and Morgan, M. 2009. Regression of post-orthodontic lesions by a remineralising cream. *Journal of Dental Research*. 88(12), pp. 1148-1153.
- Bataineh, M., Malinowski, M., Duggal, M., and Tahmassebi, J. 2017. Comparison of the newer preventive therapies on remineralisation of enamel *in vitro*. *Journal of Dentistry*. 66, pp. 37-44.
- Basappa, N., Prabhakar, A. and Manojkumar, J. 2013. *In vitro* remineralisation of enamel subsurface lesions and assessment of dentine tubule occlusion from NaF dentifrices with and without calcium. *Journal of Indian Society of Pedodontics and Preventive Dentistry*. 31(1), pp. 29-35.
- Beerens, M., van der Veen, M., van Beek, H. and ten Cate, J. 2010. Effects of casein phosphopeptide amorphous calcium fluoride phosphate paste on white spot lesions and dental plaque after orthodontic treatment: a 3-month follow-up. *European Journal of Oral Sciences*. 118(6), pp. 610-617.
- Benedict, H. 1929. The fluorescence of teeth as another method of attack on the problem of dental caries. *Journal of Dental Research*, 9, pp. 274-279.
- Bowen, W. 1995. The role of fluoride toothpaste in the prevention of dental caries. *Journal of the Royal Society of Medicine*. 88(9), pp. 505-507.
- Brochner, A., Christensen, C., Kristensen, B., Tranaeus, S., Karlsson, L., Sonnesen, L. and Twetman, S. 2011. Treatment of post-orthodontic white spot lesions with casein phosphopeptide-stabilised amorphous calcium phosphate. *Clinical Oral Investigation*. 15(3), pp. 369-373.

- Buchalla, W. 2005. Comparative fluorescence spectroscopy shows differences in noncavitated enamel lesions. *Caries Research*. 39(2), pp. 150-156.
- Burt, B. A. and Eklund, S. A. 1999. *Dentistry, dental practice, and the community*. 5th edition. Philadelphia, Pennsylvania: WB Saunders.
- Buzalaf, M. 2011. Fluoride and the oral environment. *Monographs in Oral Science*. 22, pp. 66-96.
- Buzalaf, M., Hannas, A., Magalhaes, A., Rios, D., Honorio, H. and Delbem, 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(4), pp. 316-334.
- Chapla, H., Shah, N., Parekh, V., Shah, R. and Patel, J. 2013. Comparative evaluation of efficacy of CPP-ACPF and Clinpro on enamel remineralisation with the help of DIAGNOdent- An *in-vitro* study. *International Journal of Biomedical and Advance Research*. 4(6), pp. 397-402.
- Cochrane, N. J., Zero, D. and Reynolds, E. 2012. Remineralisation models. *Advanced Dental Research*. 24(2), pp. 129-132.
- Cochrane, N. J., Cai, F., Huq, N., Burrow, M. and Reynolds, E. 2010. New approaches to enhanced remineralisation of tooth enamel. *Journal of Dental Research*. 89(11), pp. 1187–1197.
- Cutress, T., Howell, P., Finidori, C. and Abdullah, F. 1992. Caries Preventive Effect of High Fluoride and Xylitol Containing Dentifrices. *Journal of Dentistry for Children*. 59(4), pp. 313–318.
- Damato, F. and Stephen, K. 1994. Demonstration of a fluoride dose response with an *in situ* single-section dental caries model. *Caries Research*. 28(4), pp. 277-283.

- Darling, A. 1961. The structure of the enamel revealed in dental disease. *Archives of Oral Biology*. 4, pp. 80-85.
- Darling, A. 1956. Studies of the early lesion of enamel caries with transmitted light, polarised light and radiography. *British Dental Journal*. 101, pp. 289–297.
- Davies, G. 2008. High Fluoride Toothpastes: Their Potential Role in a Caries Prevention Programme. *Dental Update*. 35, pp. 320-323.
- Davies, G. Worthington, H. and Clarkson, J. 2001. The use of fibre-optic transillumination in general dental practice. *British Dental Journal*. 191(3), pp. 145–147.
- Diniz, M., Boldieri, T., Rodrigues, J., Santos-Pinto, L., Lussi, A. and Cordeiro, R. C. 2012. The performance of conventional and fluorescence-based methods for occlusal caries detection. *Journal of the American Dental Association*. 143(4), pp. 339-350.
- Dirks, O. 1966. Post-eruptive changes in dental enamel. *Journal of Dental Research*. 45, pp. 503-511.
- Driscoll, C. O., Dowker, S. E., Anderson, P., Wilson, R. M. & Gulabivala, K. 2002. Effects of sodium hypochlorite solution on root dentine composition. *Journal of Materials Science: Materials in Medicine*. 13, pp. 219-223.
- EAPD Policy Document. 2009. Guidelines on the use of fluoride in children. *European Archives of Paediatric Dentistry*. 10(3), pp. 129-135.
- Edmunds, D., Whittaker, K. and Green, R. 1988. Suitability of human, bovine, equine, and bovine tooth enamel for studies of artificial bacterial carious lesions. *Caries Research*. 22, pp. 327-336.
- Elsayed, I., Sakr, A. and Badr, Y. 2009. Combining casein phosphopeptide-amorphous calcium phosphate with fluoride: synergistic

remineralisation potential of artificially demineralised enamel or not.

*Journal of Biomedical Optics*. 14(4), pp. 1-6.

- Featherstone, J. 2004. The continuum of dental caries – evidence for a dynamic disease process. *Journal of Dental Research*. 83, pp. 39-42.
- Featherstone, J. 2000. The science and practice of caries prevention. *Journal of the American Dental Association*. 131, pp. 887-899.
- Featherstone J. 1999. Prevention and reversal of dental caries: Role of low level fluoride. *Journal of Community Dentistry and Oral Epidemiology*. 27, pp. 31-40.
- Featherstone, J. 1990. Dependence of *in vitro* demineralisation of apatite and remineralisation of dental enamel on fluoride concentration. *Journal of Dental Research*. 69, pp. 620–625.
- Featherstone, J. and Mellberg, J. 1981. Relative rates of progress of artificial carious lesions in bovine and human enamel. *Caries Research*. 15, pp. 109-114.
- Feng, Y., Yin, W., Hu, D., Zhang, Y., Ellwood, R. and Pretty, A. 2007. Assessment of auto fluorescence to detect the remineralization capabilities of sodium fluoride, monofluorophosphate and non-fluoride dentifrices. A single-blind cluster randomized trial. *Caries Research*. 41, pp. 358–364.
- Galhotra, V., Sofat, A., Dua, H. and Rohila, S. 2014. Anticariogenic and cariostatic potential of components of diet: A review. *Indian Journal of Dental Sciences*. 6(4), pp. 79-85.
- Gerould, C. 1945. Electron microscope study of the mechanism of fluorine deposition in teeth. *Journal of Dental Research*. 24, pp. 223-233.
- Gomez, J., Zakian, C., Salsone, A., Pinto, S., Taylor, A. Pretty, A. and Ellwood, A. 2013. *In vitro* performance of different methods in detecting occlusal caries lesions. *Journal of Dentistry*. 41, pp. 180-186.

- Greene, W. and Newbrun, E. 1986. A theoretical study of *in vivo* lesion repair using a controlled-release device. *Journal of Dental Research*. 65, pp. 116-119.
- Hamilton, J. 2005. Should a dental explorer be used to probe suspected carious lesion? Yes – an explorer is a time-tested tool for caries detection. *Journal of the American Dental Association*. 136, pp.1526-1530.
- Hamilton, I. 1990. Biochemical effects of fluoride on oral bacteria. *Journal of Dental Research*. 69, pp. 660-667.
- Hankin, J. H., Chung, C. S. and Kau, M. C. 1973. Genetic and epidemiologic studies of oral characteristics in Hawaii's schoolchildren: dietary patterns and caries prevalence. *Journal of Dental Research*. 52(5), pp. 1079–1086.
- Hegde, M. Shetty, S. and Pardal, D. 2007. Remineralisation of enamel subsurface lesions using casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) - quantitative energy dispersive X-ray analysis. *Journal of Conservative Dentistry*. 10, pp. 19-25.
- Heinrich-Weltzien, R., Kuhnisch, J., van der Veen, M., Josselin de Jong, E. and Stosser, L. 2003. Quantitative light-induced fluorescence- a potential method for the dental practitioner. *Journal of Quintessence International*. 34 (3), pp. 181-189.
- Hicks, M. and Flaitz, C. 2006. Amorphous calcium phosphate- casein phosphopeptide paste: Effect on enamel caries formation. *Journal of Dental Research*. 85, pp. 45-49.
- Higham, S. M., Pretty, I., Edgar, W. and Smith, P. 2005. The Use of *in situ* models and qIc for the study of coronal caries. *Journal of Dentistry*. 33, pp. 235-241.
- Hintze, H and Wenzel, A. and Danielsen, B. 1998. Reliability of visual examination, fibre-optic transillumination, and bite-wing radiography, and

reproducibility of direct visual examination following tooth separation for the identification of cavitated carious lesions in contacting approximal surfaces. *Caries Research*. 32(3), pp. 204–209.

- Hipp N., Groves, M. and McMeekin, T. 1952. Acid-base titration, viscosity and density of  $\alpha$ ,  $\beta$  and  $\gamma$  casein. *Journal of the American Chemical Society*. 74, pp. 4822-4826.
- Jayarajan, J., Janardhanam, P., Jayakumar, P. and Deepika. 2011. Efficacy of CPP-ACP and CPP-ACPF on enamel remineralisation - An *in vitro* study using scanning electron microscope and DIAGNOdent. *Indian Journal of Dental Research*. 22(1), pp. 77-82.
- Jindal, S., Gupta, N., Gupta, P., Arora, V. and Mehta, N. 2015. Reverse the adverse: A review. *International Journal of Advanced Health Sciences*. 1 (10), pp. 21-24.
- Josselin de Jong, E., Sundstrom, F., Westerling, H., Tranaeus, S., Ten Bosch, J. and Angmar-Mansson, B. 1995. A new method for *in vivo* quantification of changes in initial enamel caries with laser fluorescence. *Caries Research*. 29, pp. 2–7.
- Karlinsey, R., Mackey, A., Walker, E., Amaechi, B., Karthikeyan, R., Najibfard, K. and Pfarrer, A. 2010. Remineralisation potential of 5,000 ppm fluoride dentifrices evaluated in a pH cycling model. *Journal of Dentistry and Oral Hygiene*. 2(1), pp. 1-6.
- Karlsson, L. 2010. Caries detection methods based on changes in optical properties between healthy and carious tissue. *International Journal of Dentistry*. 10, pp. 1-9.
- Kawasaki, K. 1989. Effect of carboxymethylcellulose on fluoride uptake and remineralization in demineralized enamel. *Journal of Dental Health*. 39, pp. 242-247.

- Koulourides, T., Phantumvanit, P., Munksgaard, E. and Housch, T. 1974. An intraoral model used for studies of fluoride incorporation in enamel. *Journal of Oral Pathology*. 3, pp. 185–196.
- Krobicka, A., Bowen, W., Pearson, S. and Young, D. 1987. The effects of cheese snacks on caries in desalivated rats. *Journal of Dental Research*. 66, pp. 1116-1125.
- Kuhnisch, J., Ifland, S., Tranaeus, S., Hickel, R., Stosser, L. and Heinrich-Weltzien, R. 2007. *In vivo* detection of non-cavitated caries lesions on occlusal surfaces by visual inspection and quantitative light-induced fluorescence. *Journal of Acta Odontologica Scandinavica*. 65, pp. 183-188.
- Kuhnisch, J and Heinrich-Weltzien, R. 2004. Quantitative light-induced fluorescence-a literature review. *International Journal of Computerised Dentistry*. 7(4), pp. 325-338.
- Lagerweij, M., van der Veen, M., Ando, M., Lukantsova, L. and Stookey, G. 1999. The validity and repeatability of three light-induced fluorescence systems: an *in vitro* study. *Caries Research*. 33, pp. 220-226.
- Lammers, P., Borggreven, J. and Driessens, F. 1990. Influence of fluoride on *in vitro* remineralization of artificial subsurface lesions determined with a sandwich technique. *Caries Research*. 24, pp. 81-89.
- Lata, S., Varghese, N., and Varughese, J. 2010. Remineralization potential of fluoride and amorphous calcium phosphate-casein phosphopeptide on enamel lesions: An *in vitro* comparative evaluation. *Journal of Conservative Dentistry*. 13(1), pp. 42–46.
- Lee, Y. Lu, H. and Powers, M. 2005. Fluorescence of layered resin composites. *Journal of Esthetical Restorative Dentistry*. 17, pp. 93–100.
- Lenander-Lumikari, M., & Loimaranta, V. 2000. Saliva and dental caries. *Advanced Dental Research*. 14, pp. 40-47.

- Li, X., Wang, J., Joiner, A. and Chang, J. 2014. The remineralisation of enamel: A review of the literature. *Journal of Dentistry*. 42 (1), pp. 12-20.
- Lynch, R., Mony, U. and ten Cate, J. 2007. Effect of lesion characteristics and mineralizing solution type on enamel remineralization *in vitro*. *Caries Research*. 41, pp. 257-262.
- Lynch, R. and ten Cate, J. 2006. The effect of lesion characteristics at baseline on subsequent de- and remineralisation behaviour. *Caries Research*. 40, pp. 530-535.
- Lynch, R., Navada, R. and Walia, R. 2004. Low-Levels of fluoride in plaque and saliva and their effects on the demineralisation and remineralisation of enamel; role of fluoride toothpastes. *International Dental Journal*. 54(5), pp. 304-313.
- Marinho, V., Worthington, H., Walsh, T. and Clarkson, J. 2013. Fluoride varnishes for preventing dental caries in children and adolescents (Review). *The Cochrane Library*. CD002279.
- Marinho, V., Higgins, J., Sheiham, A. and Logan, S. 2003. Fluoride toothpastes for preventing dental caries in children and adolescents. *Cochrane Database Systematic Review*. CD002278.
- Marinho, V., Higgins, J., Logan, S. and Sheiham, A. 2002. Fluoride gels for preventing dental caries in children and adolescents. *Cochrane Database Systematic Review*.2: CD002280.
- Marsh, P. 1995. The role of microbiology in models of dental caries. *Advances in Dental Research*. 9(3), pp. 208-213.
- Marya, C. and Dahiya, V. 2006. Fluoride varnish: a useful dental public health tool. *The Internet Journal of Dental Science*. 4(2), pp. 1-4.
- McDougall, W. 1977. Effect of milk on enamel demineralisation and remineralisation *in vitro*. *Caries Research*. 11, pp. 166-172.



- Mehta, R., Nandlal, B. and Prashanth, S. 2013. Comparative evaluation of remineralisation potential of casein phosphopeptide-amorphous calcium phosphate and casein phosphopeptide-amorphous calcium phosphate fluoride on artificial enamel white spot lesion: an *in vitro* light fluorescence study. *Indian Journal of Dental Research*. 24(6), pp. 681-690.
- Mellberg, J. 1992. Hard-tissue substrates for evaluation of cariogenic and anti-cariogenic activity *in situ*. *Journal of Dental Research*. 71, pp. 913-919.
- Memarpour, M., Fakhraei, E., Dadaein, S. and Vossoughi, M. 2015. Efficacy of fluoride varnish and casein phosphopeptide-amorphous calcium phosphate for remineralisation of primary teeth: a randomised clinical trial. *Journal of Medical Principles and Practice*. 24, pp. 231-237.
- Meyer-Lueckel, H., Wierichs, R., Schellwien, T. and Paris, S. 2015. Remineralising efficacy of a CPP-ACP cream on enamel caries lesions *in situ*. *Caries Research*. 49, pp. 56-62.
- Mor, B., and Rodda, J. 1983. *In vitro* remineralisation of artificial caries-like lesions with milk. *New Zealand Dental Journal*. 79, pp. 10-15.
- Moslemi, M., Sattari, M., Kooshki, F. Fotuhi, F. Modarresi, N., Sadrabad, Z. and Shadkar, M. 2015. Relationship of salivary lactoferrin and lysozyme concentrations with early childhood caries. *Journal of Dental Research*. 9(2), pp. 109-114.
- Nakata, K., Nikaido, T., Ikeda, M., Foxton, R., and Tagami, J. 2009. Relationship between fluorescence loss of QLF and depth of demineralization in an enamel erosion model. *Dental Materials Journal*. 28(5), PP. 523–529.
- Neuhaus, K., Ellwood, R., Lussi, A., Pitts, N. 2009. Traditional lesion detection aids; Detection, assessment, diagnosis and monitoring of caries. *Monographs in Oral Science*. pp 42–51.

- Nordstrom, A. and Birkhed, D. 2010. Preventive effect of high-fluoride dentifrice (5,000 ppm) in caries-active adolescents: a 2-year clinical trial. *Caries Research*. 44, pp. 323-331.
- Oliveira, G., Ritter, A. Heymann, H. Swift, E., Donovan, T., Brock, G. and Wright, T. 2014. Remineralisation effect of CPP-ACP and fluoride for white spot lesions *in vitro*. *Journal of Dentistry*. 42, pp. 1592-1602.
- Pessan, J.P., Toumba, K. and Buzalaf, M. 2011. Topical use of fluorides for caries control. *Monographs in Oral Science*. 22, pp.115-132.
- Petersen, P. E. and Lennon, M. A. 2004. Effective use of fluoride for the prevention of dental caries in the 21<sup>st</sup> century: the WHO approach. *Journal of Community Dentistry and Oral Epidemiology*. 32, pp. 319-321.
- Pitts, N. and Ekstrand, K. 2013. International caries detection and assessment system (ICDAS) and its international caries classification and management system (ICCMS) – methods for staging of the caries process and enabling dentists to manage caries. *Journal of Community Dentistry and Oral Epidemiology*. 41, pp. 41-52.
- Pitts, N. 2004. "ICDAS"--an international system for caries detection and assessment being developed to facilitate caries epidemiology, research and appropriate clinical management. *Journal of Community Dental Health*. 21(3), pp. 193-201.
- Pitts, N. 2001. Clinical diagnosis of dental caries: European perspective. *Journal of Dental Education*. 65(10), pp. 972–978.
- Pretty, A. 2006. Caries detection and diagnosis: novel technologies. *Journal of Dentistry*. 34, pp. 727-739.
- Pretty, A., Edgar, W. and Highman, S. 2002. Detection of *in vitro* demineralisation of primary teeth using light-induced fluorescence (QLF). *International Journal of Paediatric Dentistry*. 12(3), pp. 158-167.

- Pretty, A., Hall, P., Smith, W., Edgar, M. and Higham, S. 2002. The intra- and inter-examiner reliability of quantitative light-induced fluorescence (QLF) analyses. *British Dental Journal*. 193(2), pp. 105-109.
- Prusiner, S. 1982. Novel proteinaceous infectious particles cause scrapie. *Science*. 216, pp. 136–144.
- Reynolds, E. 2008. Calcium phosphate-based remineralisation systems: Scientific evidence. *Australian Dental Journal*. 53, pp. 268-273.
- Reynolds, E. and Walsh, L. 2005. Additional aids to the remineralisation of tooth structure. *Preservation and restoration of tooth structure. Knowledge Books and Software*. 111-118.
- Reynolds, E. Cain, C. Webber, F. Black, C., Riley, P., Johnson, I. and Perich, J. 1995. Anticariogenicity of calcium phosphate complexes of tryptic casein phosphopeptide in the rats. *Journal of Dental Research*. 74, pp. 1272-1279.
- Reynolds, E. and Rio, A. 1984. Effect of casein and whey-protein solutions on caries experience and feeding patterns of the rat. *Archives of Oral Biology*. 29(11), pp. 927-933.
- Reynolds, E. and Johnson, I. 1981. Effect of milk on caries incidence and bacterial composition of dental plaque in the rat. *Archives of Oral Biology*. 26, pp. 445- 451.
- Ricketts, D., Whaites, E., Kidd, E., Brown J., and Wilson, R. 1997. An evaluation of the diagnostic yield from bitewing radiographs of small approximal and occlusal carious lesions in a low prevalence sample in vitro using different film types and speeds. *British Dental Journal*. 25; 182(2), pp. 51-59.
- Ricketts, D., Kidd, E., Smith, B., and Wilson, R. 1995. Clinical and radiographic diagnosis of occlusal caries: a study *in vitro*. *Journal of Oral Rehabilitation*. 22(1), pp. 15-20.

- Roberts. A.J. 1995. Role of models in assessing new agents for caries prevention. *Advances in Dental Research*. 9(3), pp. 312-319.
- Robinson, C., Shore, R., Brookes, S., Strafford, S., Wood, S. and Kirkham, J. 2000. The chemistry of enamel caries. *Critical Reviews of Oral Biology and Medicine*. 11, pp. 481-495.
- Roopa, K., Pathak, S., Poornima, P. and Neena, I. 2015. White spot lesions: a literature review. *International Journal of Paediatric Dentistry*. 3(1), pp. 1-7.
- Rosen, S., Min, D. Harper, D., Beck, E. and Beck, F. 1984. Effect of cheese, with and without sucrose, on dental caries and recovery of *Streptococcus Mutans* in rats. *Journal of Dental Research*. 63(6), pp. 894-896.
- Schneiderman, A. Elbaum, M. and Shultz, T. 1997. Assessment of dental caries with digital imaging fiber-optic transillumination (DIFOTI): *In vitro* study. *Caries Research*. 31(2), pp. 103–110.
- Secilmis, A., Dilber, E., Ozturk, N. and Yilmaz, F. 2013. The effect of storage solutions on mineral content of enamel. *Materials Sciences and Applications*. 4, pp. 439-445.
- Sewon, L., and Makela, M. 1990. A study of the possible correlation of high salivary calcium levels with periodontal and dental conditions in young adults. *Archives of Oral Biology*. 35, pp. 211–213.
- Shaw, J., Ensfield, B., and Wollman, D. 1959. Studies on the relation of dairy products to dental caries in caries-susceptible rats. *Journal of Nutrition*. 67, pp. 253-273.
- Shellis, R.P. 1978. A synthetic saliva for cultural studies of dental plaque. *Archives of Oral Biology*. 23(6), pp. 485-489.
- Shen, P., Manton, D., Cochrane, N., Walker, G., Yuan, Y., Reynolds, C. and Reynolds, E. 2011. Effect of added calcium phosphate on enamel

remineralisation by fluoride in a randomised controlled *in situ* trial. *Journal of Dentistry*. 39, pp. 518-525.

- Shi, X., Tranaeus, S. and Angmar-Mansson, B. 2001. Comparison of QLF and DIAGNOdent for quantification of smooth surface caries. *Caries Research*. 35, pp. 21–26.
- Silverstone, L. 1973. Structure of carious enamel, including the early lesion. *Journal of Oral Science Review*. 3, pp. 100-160.
- Sim, C., Wee, J., Xu, Y., Cheung, Y., Soong, Y., and Manton, D. 2015. Anticaries effect of CPP-ACP in irradiated nasopharyngeal carcinoma patients. *Journal of Clinical Oral Investigations*. 19, pp. 1005-1011.
- Skucha-Nowak, M., Gibas, M., Tanasiewicz, M.; Twardawa, H., Szklarski, T. 2015. Natural and controlled demineralization for study purposes in minimally invasive dentistry. *Advances in Clinical and Experimental Medicine*. 24, pp. 891–898.
- Sonesson, M., Twetman, S. and Bondemark, L. 2014. Effectiveness of high-fluoride toothpaste on enamel demineralization during orthodontic treatment – a multicenter randomized controlled trial. *European Journal of Orthodontics*. 36, pp. 678-682.
- Stookey G. 2008. The Effect of saliva on dental caries. *Journal of the American Dental Association*. 139(5), pp. 11-17.
- Stookey, G. 2005. Should a dental explorer be used to probe suspected carious lesions? No-use of an explorer can lead to misdiagnosis and disrupt remineralization. *Journal of the American Dental Association*. 136(11), pp. 1526-1532.
- Stookey, G. 2004. Optical methods – quantitative light fluorescence. *Journal of Dental Research*. 83, pp. 84-88.

- Stookey, G., Warrick, J., Miller, L. and Greene, A. 1995. Animal caries models for evaluating fluoride dentifrices. *Advances in Dental Research*. 9(3), pp. 198-207.
- Sullivan, R. J., Masters, J., Cantore, R., Petrou, I., Stranick, M., Goldman, H., Guggenheim, B. and Gaffar, A. 2001. Development of an enhanced anticaries efficacy dual component dentifrice containing sodium fluoride and dicalcium phosphate dihydrate. *American Journal of Dentistry*. 14, pp. 3–11.
- Ten Bosch, J. 1996. Light scattering and related methods in caries diagnosis. Proc. of the 1st Annual Indiana Conference. 81-90.
- Ten Cate, J., Buijs, M., Miller, C. and Exterkate, R. 2008. Elevated fluoride products enhance remineralization of advanced enamel lesions. *Journal of Dental Research*. 87, pp. 943–947.
- Ten Cate, J., Exterkate, R. and Buijjs, M. 2006. The relative efficacy of fluoride toothpastes assessed with pH cycling. *Caries Research*. 40, pp. 136-141.
- Ten Cate, J. and Duijsters, P. 1983. Influence of fluoride in solution on tooth demineralization. *Caries Research*. 17, pp. 513-519.
- Ten Cate, J. and Duijsters, P. 1982. Alternating demineralisation and remineralisation of artificial enamel lesions. *Caries Research*. 16(3), pp. 201-211.
- Ten Cate, J., Jongebloed, W. and Arends, J. 1981. Remineralization of artificial enamel lesions *in vitro*. IV. Influence of fluorides and diphosphonates on short- and long-term remineralisation. *Caries Research*. 15, pp. 60–69.
- Titley, K., Chernecky, R., Rossouw, P. and Kulkarni, G. 1998. The effect of various storage methods and media on shear-bond strengths of dental composite resin to bovine dentine. *Archives of Oral Biology*. 43(4), pp. 305-311.

- Tosun, G., Sener, Y. and Sengun, A. 2007. Effect of storage duration/solution on microshear bond strength of composite to enamel. *Journal of Dental Materials*. 26(1), pp. 116-121.
- Tubert-Jennin, S., Tramini, P., Gerbaud, L., Amsallem, E., Schulte, A., Auclair, C. and Ismail, A. 2011. Fluoride supplements (tablets, drops, lozenges or chewing gums) for preventing dental caries in children (review). *The Cochrane Collaboration*. CD007592.
- Twetman, S. 2009. Caries prevention with fluoride toothpaste in children: An update. *European Archives of Paediatric Dentistry*. 10 (3), pp. 162-169 7.
- Twetman, S., Axelsson, S., Dahlgren, H., Holm, A., Kallestal, C., Lagerlof, F., Lingstrom, P., Mejare, I., Nordenram, G., Norlund, A., Petersson, L. and Soder, B. 2003. Caries-preventive effect of fluoride toothpaste: A systematic review. *Journal of Acta Odontologica Scandinavica*. 61 (6), pp. 347-355.
- Young, D. and Featherstone, J. 2005. Digital imaging fiber-optic trans-illumination, f-speed radiographic film and depth of approximal lesions. *Journal of the American Dental Association*. 136(12), pp. 1682–1687.
- Young, D. 2002. New caries detection technologies and modern caries management: merging the strategies. *General Dentistry*. 50(4), pp. 320-331.
- Vanichvatana and Auychai. 2013. Efficacy of two calcium phosphate pastes on the remineralisation of artificial caries: A randomised controlled double-blind *in situ* study. *International Journal of Oral Science*. 5, pp. 224–228.
- Walsh, L. J. 2009. Contemporary technologies for remineralisation therapies: A review. *Journal of International Dentistry South Africa*. 11(6), pp. 6-16.

- White, D. 1995. The application of *in vitro* models to research on demineralization and remineralization of the teeth. *Advances in Dental Research*. 9, pp. 175-193.
- White, D. 1992. The comparative sensitivity of intra-oral, *in vitro*, and animal models in the "profile" evaluation of topical fluorides. *Journal of Dental Research*. 71, pp. 884-894.
- White, D. J. 1987. Use of synthetic polymer gels for artificial carious lesion preparation. *Caries Research*. 21, pp. 228-242.
- Whitford, G., Birdsong-Whitford, N. and Finidori, C. 1990. Acute oral toxicity of sodium fluoride and monofluorophosphate separately or in combination in rats. *Caries Research*. 24, pp. 121-126.
- World Health Organisation. 2006. Fluoride in drinking water. TJ International (Ltd), Padstow, Cornwall, UK.
- Wu, G., Liu, X. and Hou, Y. 2010. Analysis of the effect of CPP-ACP tooth mousse on enamel remineralisation by circularly polarized images. *Angel Orthodontist*. 80(5), pp. 933-938.
- Zero, D. T. 2006. Dentifrices, mouthwashes, and remineralisation/caries arrestment strategies. *Journal of BMC Oral Health*. 6(1), pp. 9-22.
- Zero, D. T. 1995. *In situ* caries models. *Advances in Dental Research*. 9(3), pp. 214-230.
- Zhang, Q., Zou, J., Yang, R. and Zhou, X. 2011. Remineralisation effects of casein phosphopeptide-amorphous calcium phosphate crème on artificial early enamel lesions of primary teeth. *International Journal of Paediatric Dentistry*. 21, pp. 374 - 381.
- Zhou, C., Zhang, D., Bai, Y. and Li, S. 2014. Casein phosphopeptide–amorphous calcium phosphate remineralisation of primary teeth early enamel lesions. *Journal of Dentistry*. 42, pp. 21-29.




- Ziskind, D., Gleitman, J., Rotstein, I. and Friedman, M. 2003. Evaluation of cetylpyridinium chloride for infection control in storage solution. *Journal of Oral Rehabilitation*. 30(5).pp. 477-481.

## APPENDICES

### Appendix 1: Approval for collection of bovine teeth.

F.1  
 107301304400420  
 01100700170

### Dispatch of SRM for Veterinary or Research Purposes



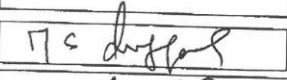
Food Standards Agency  
food.gov.uk

Approval No. <u>2091</u>	Establishment Name <u>Spensorough abattoir</u>	Date <u>11/03/11</u>
--------------------------	--	----------------------

**Part 1**  
To be completed by the person responsible for the handling and disposal of SRM

Name and address of research establishment	Professor M S Duggal, Leeds Dental Institute, University of Leeds, Clarendon Way, Leeds LS29LU Postcode: <u>LS29LU</u>		
Reason for application	Collection of bovine teeth for dental research with ethical approval. All bovine enamel is sterilised using both gamma irradiation and overnight suspension in 2.5% sodium hypochlorite 20,000ppm.		
Type of SRM tissue	Bovine incisor teeth from animal less than 13 months of age		
Quantity of SRM required	<u>100 teeth</u>	Frequency of collection	<u>monthly</u>
Duration of project From:	<u>11/10/11</u>	To:	<u>11/10/12</u>
Name and address of abattoir	Spensorough Abattoir, Headlands Road, Liversedge WF15 6PR, Dr Istvan Poc (veterinary surgeon) Postcode: <u>WF156PR</u>		
Approval number	<u>2091</u>		


**Declaration**  
I, the undersigned, declare that the SRM collected from the above abattoir will be handled, transported and disposed of in accordance with the Animal By-Products (Enforcement) Regulations 2011 (England, Scotland, Wales)

Name in BLOCK letters: <u>M S DUGGAL</u>	Position: <u>Prof &amp; Head</u>
Signature: 	Date: <u>11/03/11</u>
Email Address: <u>m.s.duggal@leeds.ac.uk</u>	

**Send this form to the Veterinary Manager at FSA York**

**Part 2**  
To be completed by the Veterinary Manager at FSA York.

I, the undersigned, have approved the application stated above. The OV at the named establishment may release SRM to the applicant in accordance with the SRM Regulations.

Name in BLOCK letters: <u>ASIER PRAZANOVA</u>	Designation: <u>VETERINARY MANAGER</u>
Signature: 	Date: <u>03/10/2011</u>

The completed form should be retained by the issuing Veterinary Manager. Copies should be sent to:

- the applicant via email or fax;
- the OV at the named establishment;
- the Animal Health Divisional Office in whose area the consignee establishment is situated;
- the Local Authority in whose area the recipient establishment is situated.

Part 3 overleaf...

(Rev 04/11) ABP 7/1

## Appendix 2: Normality tests for $\Delta F$ at baseline

### Shapiro-Wilk and Kolmogorov-Smirnov Tests of Normality for $\Delta F$ -Baseline.

Groups	Kolmogorov-Smirnov <sup>a</sup>			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
0 ppm F	0.089	26	0.200	0.951	26	0.241
1450 ppm F	0.092	26	0.200	0.970	26	0.611
5000 ppm F	0.125	26	0.200	0.946	26	0.187
1450 ppm F + Tooth Mousse	0.101	26	0.200	0.949	26	0.221
1450 ppm F + MI Paste Plus	0.088	26	0.200	0.976	26	0.784
Clinpro Tooth Crème	0.121	26	0.200	0.958	26	0.350
Clinpro 5000	0.148	26	0.150	0.947	26	0.200

### Appendix 3: Descriptive statistics for $\Delta F$ -Difference for all groups

Groups	N	Minimum	Maximum	Mean	Median	Std. Deviation
0 ppm F	26	2.500	21.667	11.78	10.6	5.86
1450 ppm F	26	14.43	24.4	18.66	18.017	2.984
5000 ppm F	26	10.73	26.83	16.058	15.1	3.95
1450 ppm F + Tooth Mousse	26	9.0	24.367	18.318	18.383	3.291
1450 ppm F + MI Paste Plus	26	12.23	32.0	18.913	18.117	4.447
Clinpro Crème	26	10.467	25.33	19.537	19.05	3.962
Clinpro 5000	26	12.0	27.167	17.59	17.167	3.591

### Appendix 4: Normality tests for $\Delta Q$ at Baseline.

#### Shapiro-Wilk and Kolmogorov-Smirnov Tests of Normality for $\Delta Q$ -Baseline.

Groups	Kolmogorov-Smirnov <sup>a</sup>			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
0 ppm F	0.123	26	0.200	0.952	26	0.253
1450 ppm F	0.124	26	0.200	0.960	26	0.388
5000 ppm F	0.115	26	0.200	0.966	26	0.527
1450 ppm F + Tooth Mousse	0.091	26	0.200	0.974	26	0.730
1450 ppm F + MI Paste Plus	0.121	26	0.200	0.952	26	0.256
Clinpro Tooth Crème	0.100	26	0.200	0.982	26	0.918
Clinpro 5000	0.130	26	0.200	0.955	26	0.300

**Appendix 5: Descriptive statistics for the  $\Delta Q$ -Difference for  
all groups**

Groups	N	Minimum	Maximum	Mean	Median	Std. Deviation
0 ppm F	26	7625.1	118728.6	51703.25	50809.9	29532.2
1450 ppm F	26	2717.7	191979.7	93949.9	87564.2	38567
5000 ppm F	26	36663	221103.5	89867.1	80743	44857
1450 ppm F + Tooth Mousse	26	29605.8	144088	95104.4	90717.8	31020
1450 ppm F + MI Paste Plus	26	54606.1	128463	91651.7	86655	23964.5
Clinpro Crème	26	45553.5	166105.1	99559.7	94598.3	28693.4
Clinpro 5000	26	37927.7	145774.67	93279.3	90722.3	25555.5

## Appendix 6: Normality tests for Area at baseline.

### Shapiro-Wilk and Kolmogorov-Smirnov Tests of Normality for lesion Area at baseline.

Groups	Kolmogorov-Smirnov <sup>a</sup>			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
0 ppm F	0.094	26	0.200	0.971	26	0.648
1450 ppm F	0.129	26	0.200	0.953	26	0.273
5000 ppm F	0.138	26	0.200	0.956	26	0.314
1450 ppm F + Tooth Mousse	0.109	26	0.200	0.969	26	0.595
1450 ppm F + MI Paste Plus	0.082	26	0.200	0.972	26	0.668
Clinpro Tooth Crème	0.095	26	0.200	0.977	26	0.813
Clinpro 5000	0.118	26	0.200	0.975	26	0.767

**Appendix 7: Descriptive statistics for the Lesion Area-  
Difference for all groups.**

<b>Groups</b>	<b>N</b>	<b>Minimum</b>	<b>Maximum</b>	<b>Mean</b>	<b>Median</b>	<b>Std. Deviation</b>
<b>0 ppm F</b>	<b>26</b>	<b>-3534.7</b>	<b>204.7</b>	<b>-519</b>	<b>-182.67</b>	<b>828.2</b>
<b>1450 ppm F</b>	<b>26</b>	<b>-4926.3</b>	<b>-243.7</b>	<b>-1737.8</b>	<b>-1606.3</b>	<b>1318</b>
<b>5000 ppm F</b>	<b>26</b>	<b>-5250</b>	<b>29.7</b>	<b>-1519.9</b>	<b>-911.5</b>	<b>1506.4</b>
<b>1450 ppm F + Tooth Mousse</b>	<b>26</b>	<b>-3379.7</b>	<b>-88.3</b>	<b>-1313.1</b>	<b>-1386.2</b>	<b>937.8</b>
<b>1450 ppm F + MI Paste Plus</b>	<b>26</b>	<b>-3603.3</b>	<b>-68.7</b>	<b>-1520.8</b>	<b>-1193</b>	<b>1231.7</b>
<b>Clinpro Tooth Crème</b>	<b>26</b>	<b>-4504</b>	<b>-184.3</b>	<b>-1527.4</b>	<b>-1336</b>	<b>1257.6</b>
<b>Clinpro 5000</b>	<b>26</b>	<b>-4048.3</b>	<b>-291.7</b>	<b>-1144</b>	<b>-930</b>	<b>867.3</b>