INVESTIGATIONS OF THE POTENTIAL EFFECTS OF DIFFERENT PREVENTIVE PRODUCTS ON TREATMENT AND PREVENTION OF ENAMEL SURFACE LOSS

By

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Submitted in accordance with the requirements for the Degree of Doctor of Philosophy

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April 2018

"I hereby confirm that this submitted work is my own and that appropriate credit has been given where reference has been made to the work of others."

Dedicated to my family

(My husband, my children, my parents, my sisters and brothers.)

ACKNOWLEDGEMENTS

Many people deserve thanks for help while this extremely challenging task was being done. Special gratitude goes to the head of the Paediatric Dentistry Department, Professor Monty Duggal, for his continued encouragement, guidance and his generosity in offering his long experience and knowledge during my PhD study. Many thanks go to Prof. Jack Toumba for his help and support, Dr Simon Strafford for offering his previous laboratory technical experience and guidance during the initial stages of the work which contributed considerably to this thesis.

I gratefully value the comments and the scientific guidance involvement of The Head of Clinical Management trial / (Dentistry)/Clinical Trial Research / University of Leeds, Dr Catherine Fernandez, for her valued comments and scientific guided involvements during the clinical trial work. I sincerely appreciate the help and the assistance of the clinical trial study coordinator, Gillian Dukanovic, for her helpful well-organised involvement in clinical trial and Ashna Chavda for her assistance and helpful pleasant character.

I gratefully acknowledge all the volunteers who participated in this clinical trial for their lovely and cooperative characters. I acknowledge the statisticians Dr Theresa Munyombwe and the Biostatistician Jing Kang for their precious advice and guidance on the statistical analysis.

I would like to thank the Dental Laboratory Technicians for making the research appliances for the study and Ms Clare Verity for her coping with my requirements and materials for the study and my colleague Marina Malinowski who offered her help in designing the blindness of the study test products.

Special thanks go to the Libyan authorities for their scholarship provision throughout the whole period of this demanding study.

I express my very deep appreciation and gratitude to my husband, my children, my parents, my sisters and brothers for their unlimited love and everlasting support during my study life.

ABSTRACT

Maintaining strong healthy teeth for life is important; therefore the present study was concerned with the recognition of the significance of exploring some of the preventive and therapeutic products on the surface loss of enamel slabs subjected to acidic erosion and tooth brushing abrasion challenges using *in vitro* and *in situ* experiments.

Study 1 *In vitro* assessment of the effect of fluoridated toothpastes on bovine and human enamel subjected to acidic erosion and tooth brushing abrasion.

Aims: To assess the anti-erosive potential of toothpastes containing different delivery fluoride systems using bovine dental enamel (Part I) and human enamel (Part II) subjected to both acidic erosion and tooth brushing abrasion.

Part I: Methods: Fifty bovine enamel slabs were mounted in acrylic blocks, ground flat, tested for flatness by scanning profilometry (Proscan 2000, Scantron, UK), standardised for hardness using a Knoop hardness tester (under 100 g load for 15 s) and divided into five experimental groups (E1-E5) including 10 specimens per group. The tested toothpastes were : E1 Meridol® group, (AmF/SnF2; 0.14%), E2 Elmex® anti-caries group (AmF; 0.14%F), E3 Pronamel®, (NaF, 1450 ppm F), E4 Elmex® sensitive plus (AmF; 0.14%F), and E5 Aronal® 0 ppm F. De/remineralisation cycling procedures were achieved with immersion of the slabs under static conditions in aliquots of citric acid (0.3%, pH 3.6) for 2 mins followed by soaking for 1hr in artificial saliva between the erosive challenges. The erosive challenges procedures were repeated 5 times daily for 28 days. All groups were subjected twice a day to brushing abrasion during application of a slurry of toothpaste/saliva (1: 3) with 15 tooth brushing strokes using a 300 g load and incubated overnight at 37 ˚C in night time saliva at the end of the last brushing of the enamel slabs. Erosion and abrasion depths (µm) were quantified using profilometry

scanning (Proscan 2000, Scantron, UK) at 7, 14, 21 and 28 days. **Part II**: Similar erosive / abrasive experimental procedures were used on human enamel specimens. Results: After 28 days of erosion cycling with tooth brushing abrasion, all groups showed different enamel surface loss (µm). One-way ANOVA, demonstrated that E1 showed similar trends with E5, whereas (E2, E3, E4 showed significantly less surface loss ($p<0.05$) compared to E1 and E2. Conclusions: The effect of dental erosion and tooth brushing abrasion combined can be decreased by toothpastes containing amine fluoride and sodium fluoride, whereas no protection was observed with the stannous and amine fluoride paste. Similar result trends were observed for both bovine and human enamel specimens under this erosive/abrasive model.

Study 2 The effect of therapeutic products in combination on prevention of tooth surface loss.

Aims: To study combined topical fluoridated measures on erosive and abrasive enamel wear in vitro. Methods: Sixty bovine enamel specimens were divided into four groups containing (15 samples per group) were subjected to cyclic de /remineralisation procedures. Demineralisation was performed with 0.3% citric acid (pH 3.6) 2 min / five times per day. The enamel slabs were immersed in day artificial saliva between each erosive challenge for 1 hour and incubated overnight in night saliva at 37 ^{o}C. Two times daily fluoride application with toothpaste and artificial saliva slurry (1:3 ratio) plus a tested mouthrinse with automated tooth brushing (15 strokes, 300 g load) for (2x2 min/ day) during the experimental process. The test product treatments were: group 1 Elmex® sensitive plus toothpaste (AmF, 1400 ppm F) Plus Elmex® erosion mouthrinse containing (500 ppm F as AmF/NaF and 800 ppm Sn as $SnCl_2$) stannous chloride rinse (Elmex TP plus MR) 2 times / day; group 2 Pronamel® toothpaste (NaF, 1450 ppm F+ 5%KNO_3) + Pronamel® (NaF) mouthrinse (Pronamel® TP plus MR) 2 mins x2 times /

day; group 3 Pronamel® toothpaste (NaF, 1450 ppm F) 2 / day plus GC tooth mousse™ once/ a day for 5 mins (Pronamel® TP plus TM) after the last brushing; group 4 (0 ppm F toothpaste) as a control. Enamel loss was determined by scanning profilometry after the last experimental days at 7, 14, 21, and 28 days. Results: after 28 days significantly less enamel surface loss (µm) was observed with group1: (Elmex TP plus MR) (0.40±0.23) and group 2: (Pronamel TP plus MR) (0.60±0.28)(Pronamel TP plus TM) had (2.65±1.79) ($p<0.01$) compared to 0 ppm F control (1.84±1.85). Conclusions: The topical applications of preventive therapeutic measures in the form of AmF, 1400 ppm F toothpaste plus Elmex® erosion dental rinse (500 ppm F + 800 ppm Sn) or applications of NaF/5% KNO_3 toothpaste plus NaF mouthrinse significantly decreased the effect of erosion and tooth brushing abrasion compared to combinations containing remineralising agent or fluoride-free toothpaste.

Study 3: Investigations of therapeutic products on prevention of enamel surface loss under erosive and abrasive challenges *in situ*

Aims: To investigate the effect of fluoridated toothpaste alone or in combination with mouth rinse on the prevention of tooth surface loss under acidic erosion and tooth brushing abrasion *in situ*. Methods**:** Seventeen healthy participants wore a small upper removable mouth appliance holding two sterilised bovine enamel slabs that were randomly assigned to investigate one of the five test products for 14 days entered a prospective controlled, double-blind, crossover with five phases trial. All participants that signed informed consents and fulfilled the inclusion criteria were enrolled in the study. The five treatment groups were: group A (Elmex® erosion protection toothpaste (1400 ppm F as AmF/NaF and 3500 Sn_2); group B (non-fluoride® toothpaste as a control); group C (Sensodyne Pronamel® 1450 ppm F as NaF/5%KNO_3 toothpaste); group D (.Sensodyne Pronamel® 1450 ppm F as NaF toothpaste plus Pronamel®

mouth wash as 450 ppm F NaF) and group E (Elmex® erosion protection toothpaste (1400 ppm F as AmF/NaF and 3500 Sn_2) with Elmex® erosion protection dental rinse as AmF and NaF 500 ppm F plus stannous chloride 800 stannous (Sn_2). The enamel slabs were dipped extra-orally in 0.3% citric acid solution at predetermined times for 2 minutes/ five times daily. In addition the enamel slabs were brushed for 1min extra-orally then 1 min intra-orally using the standard toothpaste/natural saliva slurry and 10 ml mouthrinse was used for 60s twice per day (10 ml x2). Enamel loss was determined by surface profilometry (Proscan 2000, Scantron, UK). Results: Treatment with therapeutic products Elmex® erosion protection toothpaste, Sensodyne Pronamel® toothpaste, Sensodyne Pronamel® toothpaste plus Sensodyne Pronamel® mouthrinse and Elmex® protection erosion toothpaste and Elmex® erosion protection rinse demonstrated a highly statistically significant difference in reducing the erosive and abrasive enamel surface loss ($p \leq 0.001$) compared to the control group (0 ppm F).

Conclusion: Elmex® erosion protection (1400 ppm F) toothpaste combined with Elmex® erosion protection mouthrinse gave the greatest outstanding benefit. Furthermore, using combined anti-erosive therapeutic products in the form of Sensodyne Pronamel® 1450 ppm F NaF toothpaste plus Pronamel® mouth wash 450 ppm F NaF; Elmex® erosion protection toothpaste (1400 ppm F as AmF).and Sensodyne Pronamel® 1450 ppm F as NaF toothpaste significantly reduced the daily effect of erosive/abrasive tooth wear and provided better enamel surface loss reduction compared to the non-fluoride® toothpaste.

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LIST OF ABREVIATIONS

pH	Acidity
AE	Adverse Event
AmF	Amine Fluoride
°C	Degrees Celsius
CRF	Case Record Form
e.g.	For example
E	Experimental group
ESL	Enamel Surface Loss
Etc.	Etcetera
F	Fluoride
g	Gram(s)
GCP	Good Clinical Practice
grp	Group
≥	Greater than or equal to
H(s)	Hour(s)
≤	Smaller than or equal to
KNO3	Potassium nitrate
Max.	Maximum
µg	Microgram
µm	Micrometre
ml	Millilitre
mm	millimetre
mins	Minutes
Minim	Minimum
Mol	Mole
MR	Mouthrinse
NaF	Sodium Fluoride
ppm F	Part per million fluoride
S	Seconds
SAE	Serious Adverse Reaction
SD	Standard Deviation
SE	Standard Error
SP	Surface Profilometery
SnF2	Stannous Fluoride
TM	Tooth Mousse
TP	Toothpaste
TSL	Tooth Surface Loss
UK	United Kingdom
Vol	Volume
Wt	Weight

1. INTRODUCTION

1.1 Tooth surface loss

Tooth surface loss (TSL) or tooth wear refers to the pathological loss of tooth tissue by a disease process other than dental caries without involvement of oral bacteria (Eccles, 1978, Madlena et al., 1994). It has been described as a multifactorial processes (Meurman and ten Cate, 1996) with erosion, Attrition, abrasion and abfraction playing foremost roles in the tooth material loss (Nunn 1996). Generally, it is difficult to distinguish between these problems, but in certain conditions the site and the appearance of the dental wear may provide a clinical sign of the possible aetiological dental pathology such as tooth wear affecting the palatal surfaces and incisal edges of the maxillary dentition (Nunn, 1996, Dyer et al., 2001, Bartlett and Shah, 2006). Loss of tooth tissue as a result of surface wear is now days becoming a significant problem in clinical dental practice amongst both adults and children (Dyer et al., 2001, Bartlett, 2007, Davies and Davies, 2008) and being extensively reported (Nunn, 1996, Kreulen et al., 2010).

The pathological tooth surface loss should be differentiated from physiological tooth surface loss which can happen naturally as a compensatory phenomenon as a result of function i.e. abrasive foods or age-related which is regulated by secondary dentine formation, alveolar bone growth and muscular adaptation to improve chewing efficiency (Berry and Poole, 1976, Davies et al., 2002). The description offered by Smith and Knight could help in differentiation between pathologic and physiologic wear—“Tooth wear can be regarded as pathological if the teeth become so worn that they do not function effectively or seriously mar appearance before they are lost

through other causes or the patient dies". The distinction between acceptable and pathological tooth wear at a given age is based on the prediction of whether the tooth will survive the rate of wear" (Smith and Knight, 1984). Tooth wear is a cumulative lifetime process, which may lead to substantial tooth surface loss (Lussi et al., 2004a). It is not unexpected to find normal "wear and tear" on the surface of the natural teeth over time due to every day function such as chewing fibrous food, drinking acidic beverages and over-enthusiastic tooth brushing but in case of pathological teeth surface loss will cause loss of function and occasional pain and hence the early recognition and prevention of pathological tooth surface loss to promote a good oral health are the useful approach during life time (Whelton, 2007).

The estimated natural physiological as a result of functional process in both vertical tooth height loss is approximately 20 – 38 µm per annum (Lambrechts et al., 1989) and also naturally occurring in the interproximal dental wear in which the horizontal tooth width undergo compensation by alveolar bone remodelling and a constant pressure forwarding to maintain proximal tooth to tooth contact (Biggerstaff, 1967, Kaidonis et al., 1992) but the pathological tooth surface loss represents an undesirable level of tooth wear (Davies et al., 2002, Bartlett and Dugmore, 2008). Several aetiological factors can lead to pathological TSL such as erosion, abrasion, attrition, abfraction and bruxism (Davies et al., 2002). Knowing the pathodynamic mechanisms, classifications, possible aetiological factors and clinical presentations of the different tooth surface lesions will facilitate making a proper differential diagnosis and providing accurate preventive and clinical treatment (Grippo et al., 2004).

1.2 Classification of tooth surface loss

TSL can be classified according to their cause into abrasion, abfraction, attrition and erosion; in addition the aetiology of the tooth surface lesions may result from any combinations of these types (ten Cate and Imfeld, 1996).

1.2.1 Erosive tooth wear

Erosion is derived from the Latin word erodere, erosi, erosum which means to corrode and describes the progressive tooth surface wear by chemical processes that does not involve bacterial action (Imfeld, 1996a, Imfeld, 2007). Although, dental erosion is extensively reported and becoming the most common pathological dental wear, moreover a wide majority of people are still unaware of the complications of erosive dental wear (Lussi et al., 2006).

1.2.2 Abrasive tooth wear

It is referred to the abnormal wearing away of the dental structure by exogenous mechanical means such as involving foreign objects of substances repeatedly introduced into the mouth and contact to the teeth such as tooth brushing and interdentally cleaning devices such as tooth picks, dental floss or brushes and abrasive toothpastes (Imfeld, 1996a, Grippo et al., 2004). Occupational or professional exposure to the abrasive particles that resulting from dust work (Meo 2004) or/ and it could be habitual due to biting on hard objects such as pens, pencils, pipe stems and fingernail biting, the use of miswak (a herbal wooden stick) or holding thread between the teeth and also clasps of partial denture (Johansson et al., 1991, Imfeld, 1996a, Grippo et al., 2004).

1.2.3 Demastication

Demastication refers to the wearing process as a result of chewing and derived from the Latin words *mandere, niandi, mansum* (to chew) and depends upon the abrasiveness of food and the masticatory force (Imfeld, 1996a, Bourdiol and Mioche, 2000). However, this is a form of physiological dental wear that after primarily affects the occlusal and incisal surfaces (Davies et al., 2002). It may be termed as a pathological condition when the cause due to frequent eating of abnormal foods such as betel nuts (Schamschula et al., 1978, Imfeld, 1996a). Masticatory abrasion may be seen in lingual and facial surfaces of the teeth as a result of coarse food contacting these surfaces during mastication (Grippo et al., 2004).

1.2.4 Attrition

The attrition wear of human teeth is referred to as the occlusal – contact – area wear as a result of tooth – to – tooth friction and the quantitative total enamel wear after 4 years for molars was suggested to be 153 µm and 88 µm on premolars in *in vivo* study (Lambrechts et al., 1989). Severe extensive wear attrition was considered as a result of masticatory stress due to lingually tilted teeth (Reinhardt, 1983). Attrition tends to be more severe in the presence of erosion and both mechanisms lead to more tooth tissue loss (Khan et al., 1998).

1.2.5 Abfraction

Abfraction is derived from the words *frangere, fregi, fractum* (to break), it is seen as a wedge-shaped defect at the cement-enamel junction caused by eccentric loading which primarily could be due to occlusal interferences, premature contacts and bruxism (Grippo et al., 2004, Imfeld, 2007).

1.3 The significance of erosive tooth surface loss in clinical dentistry

Recently, the increasing importance of erosive tooth surface loss is because it is becoming the most common pathological dental hard tissue loss resulting primarily from non – bacterial chemical attack, usually involving acidic substances. Acidic attacks lead to demineralisation and softening of tooth surfaces. As a sequel, the eroded tooth surface is more prone to abrasion and attrition and may cause real problems for the patient and the dentist (Schlueter et al., 2012). The consequences of erosive tooth wear besides the mechanical impact of tooth brushing that result in tooth surface loss presents a significant issue among various populations and most age groups (Lussi et al., 2011).

Pathological dental wear is now frequently seen in many adults who are aware of their oral health and subsequently retaining their natural teeth significantly for longer periods. Adult patients are often late in recognising that they have erosion and development of dental wear. Younger populations are increasingly affected by dental erosion due to changes in life style and increasing dietary acidic erosive drinks and consumption of food stuff.(Shaw and Smith, 1999, Lussi et al., 2011). Moreover, it was shown that pathological tooth wear in a group of 65+ year olds was three times more than people aged 26 – 35 years (Smith and Robb, 1996).

Non-carious dental mineral loss induced by direct impact of exogenous or endogenous acids result in loss of dental hard tissue, which can be serious in some groups, such as those with eating disorders, in patients with gastro-oesophageal reflux disease, and also in persons consuming high amounts of acidic drinks and foodstuffs (Montgomery et al., 1987). For these persons, erosion can impair their

well-being, due to changes in appearance and/or loss of function of the teeth, e.g., the occurrence of hypersensitivity of teeth if the dentine is exposed. If erosion reaches an advanced stage, time and money consuming therapies may be necessary (Bartlett, 2007). The therapy, in turn, poses a challenge for the dentist, particularly if the defects are diagnosed at an advanced stage. While initial and moderate defects can mostly be treated non- or minimally invasively, severe defects often require complex therapeutic strategies, which frequently entail extensive loss of dental hard tissue due to dental preparation and constructive procedures. A major goal should therefore be to diagnose dental erosion at an early stage, to avoid functional and aesthetic impairments as well as pain sensations and to ensure longevity of the dentition (Schlueter et al., 2012).

It was found that more than three-quarters (77%) of examined dentate adults had dental wear wherein 15% showed moderate wear and 2% had severe wear during the Adult Dental Health Survey 2009 (ADHS 2009) to detect the common oral health conditions and their impact on the population conducted in the UK in 2009/10 (White et al., 2012). Compared to the ADHS 1998 in which two-thirds (66%) of adults examined had wear in their anterior teeth, with 11% of adults presenting with moderate wear and 1% with severe wear. Dental wear increased with age, from 36% of 16 to 24 year olds to 89% of those aged 65 years and over.

Thus adults with both moderate and severe wear increased with age so that consequently by 65 years of age and older, one-third of dentate adults (33%) had some teeth with moderate wear and 6% had some severe form of anterior tooth wear. Regarding the severity of dental wear, moderate wear was seen in 14% of men and 8% of women; severe wear was recorded for 2% of men and less than 1% of women.

The incisal surfaces of all six upper anterior teeth displayed the highest wear (Kelly et al., 1998).

Since TSL is usually of multifactorial aetiology, hardly manifests as an isolated single occurring factor and although the raising importance of dental erosion was highlighted early in children in the United Kingdom (O'Brien et al., 1994). It was found that one-third of the toddlers and half of the teenagers exhibited some form of erosion (Nunn et al., 2003). In addition, the impact of attrition and abrasion is also recognised and the term dental wear is more commonly used (Imfeld, 2007, Bartlett and Dugmore, 2008). Therefore the present study focused on the investigation of the effect of different oral fluoridated care products on enamel surface loss due to both erosive wear and tooth brushing abrasion using *in vitro* and *in situ* models.

1.4 The aim and objectives of the present study

1.4.1 The aim

To investigate the preventive and treatment effects of different therapeutic fluoridated oral care products against acidic erosion and tooth brushing abrasion using *in vitro* and *in situ* models under controlled conditions.

1.4.2 The objectives

1. An assessment of the effects of specialised fluoridated tooth pastes on both bovine and human enamel surface loss subjected to acidic erosion and tooth brushing abrasion *in vitro*.
2. An Investigation of the effect of therapeutic products in combination on prevention of tooth surface loss *in vitro*.
3. An Investigation of therapeutic products on prevention of enamel surface loss under erosive and abrasive challenges *in situ*.

1.5 The general research questions

1. Do topical applications of different fluoridated toothpastes have similar protective effects against enamel surface loss subjected to acidic erosion and tooth brushing abrasion?

2. Do topical applications of different fluoridated toothpastes alone or in combination with mouthrinse products have similar protective effects against enamel surface loss subjected to acidic erosion and tooth brushing abrasion?

2 LITERATURE REVIEW

2.1 Erosive tooth surface loss versus dental caries

Most research studies use the clinical term dental erosion to describe the physical results of a pathologic, chronic, localised, loss of dental hard tissue that is chemically etched away from the tooth surface by acid and/or chelation without bacterial involvement (ten Cate and Imfeld, 1996).

It is a result of dissolution of tooth substances by acids when the fluid level surrounding the tooth is under-saturated with respect to tooth mineral. After an acidic challenge exposure for a sufficient time, a clinically visible defect occurs. Initially, on smooth surfaces, as a result of acid etching of the enamel and causes the original shine of the tooth to become dull as a result of destruction of the subsurface layer. Later, the convex areas flatten or shallow concavities develop and when the acidic challenge has been acting for long enough, a clinically visible defect occurs. On smooth surfaces, the original gloss of the tooth dulls. Later, the convex areas flatten or shallow concavities become present which are mostly located coronals to the enamel-cementum junction. On the occlusal surfaces, cusps become rounded or cupped and edges of restorations proud above the level of the adjacent tooth surfaces. In severe cases, the whole tooth morphological structure disappears and the vertical crown height can be significantly reduced (Lussi and Jaeggi, 2008). The result of continuing acid exposure, however, is not only a clinically visible defect, but also a change in the physical properties of the remaining tooth surface. It is recognised that erosive demineralisation results in a significant reduction in microhardness, making the softened surface more prone to mechanical impacts. Although independent in origin, erosion is therefore linked to other forms of

wear not only because it contributes to the individual overall rate of tooth tissue loss, but also by enhancing physical wear (ten Cate and Imfeld, 1996, Meurman and ten Cate, 1996, Ganss, 2006, Imfeld, 2007).

It is important to differentiate tooth damage caused by dental erosion versus damage due to caries. Both lesions are caused by acidic attack to the surface of the tooth but there are differences in the type of acid, as well as to the direction of those acids towards the specific site of the tooth surface. Although the final destructive pathology stage in both dental caries and erosion is dissolution of apatite crystals, these two pathologies differ in aetiology and histopathological mechanisms and rarely occur simultaneously. Caries is usually found under plaque – covered surfaces in which the primary acid that causes caries is the lactic acid produced as a result of fermentable carbohydrates by plaque bacteria, predominantly *S. mutans* and other cariogenic bacteria such as *Lactobacillus that* have also been involved in the initiation of dental caries (Tanzer et al., 2001, Takahashi and Nyvad, 2011). Whereas, erosion occurs on plaque-free sites and the concentration of acid is far greater in erosion than in caries, the survival activity of cariogenic bacteria can be affected with the acidity of erosive challenges at lower pH values (Silva Mendez et al., 1999). Erosive tooth wear is often seen in individuals who are conscious of their general health and well-being e.g. lacto vegetarians (Linkosalo and Markkanen, 1985, Khan et al., 1999) and conscious of their oral health (Sangnes and Gjermo, 1976). However, erosion is considered primarily a surface phenomenon while caries begins with subsurface demineralisation of enamel structure and erosive lesions are irreversible while caries lesions are reversible (ten Cate and Imfeld, 1996, Kidd and Fejereskov, 2004) (Figure 2.1)

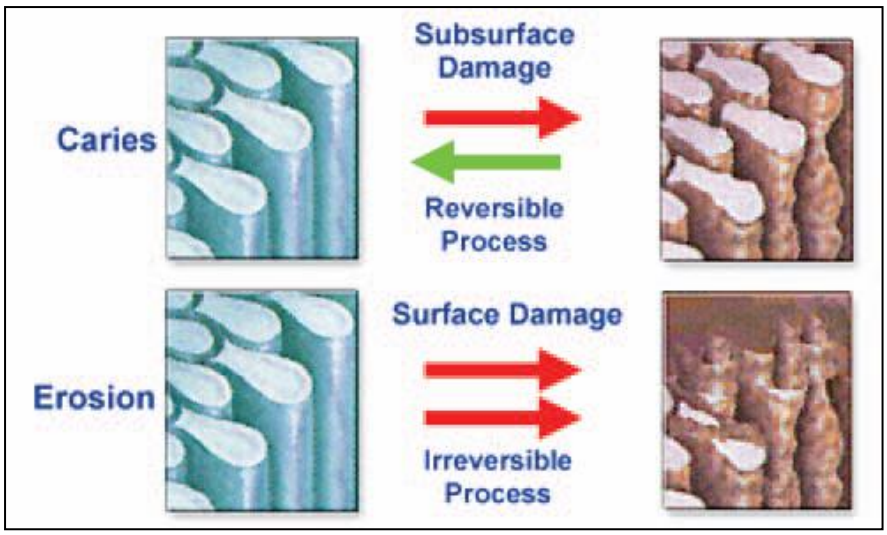


Figure 2.1 Dental erosion versus dental caries

Figure 2.1 Courtesy from Crest® Oral-B® at dentalcare.com Continuing Education Course, December 19, 2006 (Faller). Dental caries experience was associated with frequency of consumption of sugared dietary items but not with dental erosion (Huew et al., 2012).

2.2 Clinical presentations, complications and management of tooth surface loss

It is important for practicing dental professionals to recognise the features of pathological TSL and they should be able to distinguish this from physiological TSL, considering the amount of tooth wear in relation to age and in the absence of consensus and acceptable criteria for physiological TSL, pathological TSL should be considered (Davies et al., 2002). Therefore, early recognition of the clinical signs and symptoms is essential to establish the diagnosis (Lussi et al., 2006).

2.2.1 The most common pathological features of TSL

2.2.1.1 Pain and / or sensitivity to thermal stimuli

Dentine hypersensitivity resulting from exposure of underlying dentine may arise as a result of loss of enamel and or root surface exposure due to gingival recession at the cervical margin on the buccal or labial aspects of the affected teeth (Orchardson and Gillam, 2006). Enamel loss is the result of individual susceptibility to acid erosion from dietary acids from food such as citrus fruits, fruit juices, and carbonated drinks and is the major recognisable source for “softening” of the enamel surface and reducing the resistance of normal enamel to any mechanical stresses of tooth brushing with or without toothpaste. Also “softened” enamel becomes highly susceptible to any physical stresses such as abrasion, attrition or abfraction (Dababneh et al., 1999, Eisenburger et al., 2003, West, 2008). In advanced erosive cases the exposure of dentinal tubules and subsequent bacterial infiltrations will lead to sensitivity and pulpal inflammation (Brännström et al., 1967, Absi et al., 1987).

2.2.1.2 Change in appearance

TSL may affect the appearance of the front teeth and individuals with severe forms of erosion become conscious about their teeth and seeking improvement of their appearance is mainly the prime concern of the patient (Dyer et al., 2001, Davies et al., 2002, Daly et al., 2011). The appearance of a smooth silky-glazed appearance with an absence of perikymata (Figure 2.2) and intact enamel along the gingival margin, with cupping and grooving of occlusal surfaces are some typical signs of enamel and dentine erosion (Grippo et al., 2004, Imfeld, 2007). It was suggested the typical enamel band surrounds the gingival margins might be due to plaque particles, which forms as a protective layer against acids (Lussi et al., 2006).

2.2.1.3 Alveolar – occlusal vertical dimension decrease

Affecting the vertical occlusion dimension in severe dental wear which may require extensive restorative treatment such as surgical lengthening interventions of the worn crowns to enable restorations due to lack of space to ensure adjustment of freeway space and vertical dimension of occlusion (Dyer et al., 2001, Davies et al., 2002). The occlusal erosive wear is characterised by rounding of the occlusal cusps and elevated restorations above the level of the adjacent teeth surfaces and in some cases the whole occlusal surfaces have disappeared as shown in (Figure 2.4) (Lussi and Jaeggi, 2008).

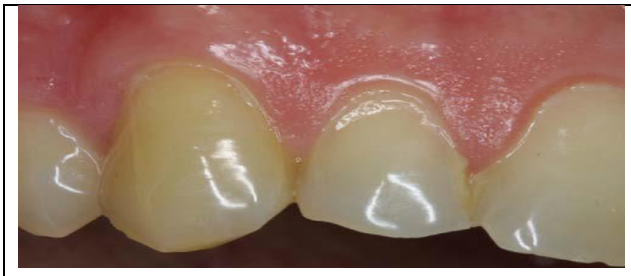


Figure 2.2 Silky smooth - glazed appearance and loss of pyrikamata of the intact enamel

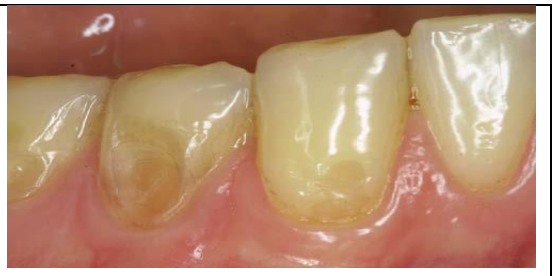


Figure 2.3 Advanced dental erosion forming concavities in the facial surfaces of the erosive teeth

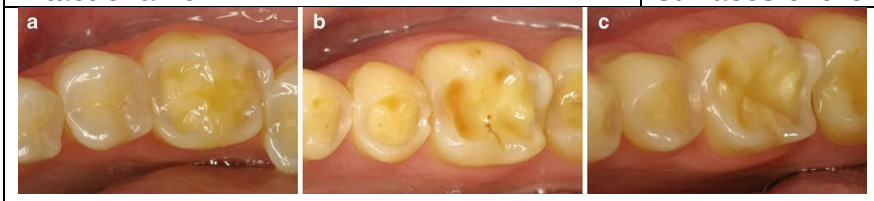


Figure 2.4 Advanced occlusal erosive wear

Courtesy of (Lussi and Jaeggi 2008)

2.3 Epidemiological indices to measure TSL

Most epidemiological dental wear indices are based on quantification of the loss of tooth surfaces by evaluation and estimation of changes in the incisal and occlusal surfaces such as the amount of enamel loss. Area of exposed enamel, and the reduction in height of the clinical crown (Smith and Knight, 1984, Dahl et al., 1993) or selected sites of the most involved segments (O'Brien et al., 1994).

Another classification uses a qualitative evaluation with an estimation of the need for treatment (øilo et al., 1987, Dahl et al., 1989). However, most disadvantages of the existing classifications (indices) are subjective evaluations and they do not provide a complete classification of the corresponding wear of restorative materials (Dahl et al., 1993) or need to be modified to assess (Donachie and Walls, 1995). Various studies have reported the prevalence of dental erosion rather than dental wear (Johansson et al., 2001).

The clinical appearance of the affected teeth and the comprehensive dietary inquiries and dental history to diagnose the aetiological risk factors are the most challenging to dental professionals (Bartlett and Shah, 2006, Bartlett and Dugmore, 2008).

In most cases changes in the morphological anatomical structure of the teeth due to dental wear are a combined process of erosion, abrasion, attrition and it is hard to consider which one is the most important (Lussi et al., 1993, Barbour and Rees, 2006). The ideal index should be simple, reproducible, easy to understand and use clinically as well as epidemiologically (Bardsley, 2008).

The most commonly used erosion indices have been described by:(Bardsley, 2008) and are:

2.3.1 The Eccles index (Eccles, 1979)

This classification (Table 2.1) was suggested during the clinical survey of 72 patients with non-industrial dental erosion and the medical history, dietary history, clinical examination, and colour photographs of the affected teeth were included in the survey (Eccles, 1979).

Table 2.1 The Eccles Index (1979)

Class	surface	Criteria
Class I	Labial	Early erosion lesion, absence of developed ridges, smooth glazed surface mainly on labial surfaces of upper incisors and canines.
Class II	Facial	Dentine involved of two types: Type 1(most common): ovoid – crescent in outline, concavity at cervical region of surface. It should be differentiated from wedge shaped abrasion lesions. Type 2: irregular lesion, entirely within crown. Punched out appearance, where enamel is absent from floor.
Class IIIa	Facial	More extensive destruction of dentine, affecting anterior teeth, Majority of lesions affect large part of the surface, but some are localised and hollowed out.
Class IIIb	Lingual or palatal	More than one third of the surface of dentine is eroded area. Gingival and proximal enamel margins have white, etch appearance. Lustrous Incisal edges due to loss of dentine. Dentine is smooth and flat or scooped out, often extending into secondary dentine.
Class IIIc	Incise or occlusal	Surfaces involved into dentine, appearing flattened or with cupping. Incisal edges appear translucent due to undermined enamel; restorations are raised above tooth surface.
Class IIId	All	Severely affected teeth, where both labial and lingual surfaces are extensively involved. Proximal surfaces may be affected; teeth are shortened.

2.3.2 The Smith and Knight Tooth Wear Index (1984)

Tooth wear index (TWI) is a further development of Eccles' idea in which all the visible surfaces of all teeth are scored (Smith and Knight, 1984) Tables 2.2 and 2.3

Table 2.2 The scoring criteria of the Smith and Knight Tooth Wear Index (1984)

Score	Surface	Criteria
0	B/L/O/I C	No loss of enamel characteristics No loss of contour.
1	B/L/O/I C	Loss of enamel characteristics Minimal loss of contour
2	B/L/O I C	Loss of enamel exposing dentine for less than one third of surface. Loss of enamel just exposing dentine. Defect less than 1 mm deep.
3	B/L/O I C	Loss of enamel exposing dentine for more than one third of surface. Loss of enamel and substantial loss of dentine. Defect less than 1 – 2 mm deep.
4	B/L/O I C	Complete enamel loss – pulp exposure – secondary dentine exposure. Pulp exposure or exposure of secondary dentine. Defect more than 2 mm deep – pulp exposure – secondary dentine exposure.

Table 2.3 The diagnostic criteria and severity grading of TWI 1984

Diagnostic criteria	severity
1. Absence of development ridges on the enamel resulting in smooth glazed enamel and in severe cases complete loss of whole enamel 2. Concavities primarily in the cervical area of labial or lingual enamel surfaces whose breadth greatly exceeds their depth, thus distinguishing them from cervical abrasion 3. Margins of amalgam and silicate restorations raised above the level of adjacent teeth Cupping of the posterior teeth cusps and grooved appearance on incisal edges.	1. Grade 0: no erosion 2. Grade 1: incipient gazed and smooth appearance. 3. Grade 2: moderate involvement of dentine. 4. Grade 3: involvement of dentine for more than one third of the area of the tooth surface.

2.3.3 **Linkosalo and Markkanen index** (Linkosalo and Markkanen, 1985).

Twenty six lacto vegetarians and their age and gender-matched controls were assessed for dental erosion using impressions and photographs. Occlusal, lingual and buccal surfaces and the gingival region were assessed (Table 2.4).

Table 2.4 Linkosalo and Markkanen index (1985)

The listed diagnostic criteria of erosion	Grading of the severity of erosion
1. Absence of developmental ridges on the enamel, resulting in a smooth glazed enamel surface. In severe cases complete loss of enamel. 2. Concavities primarily in the cervical region of the labial, or sometimes lingual, enamel surfaces whose breadth greatly exceeds their depth. 3. Edges of amalgam and silicate restorations raised above the level of the adjacent tooth surface. 4. Cupping on the cusps of posterior teeth and a grooved appearance on the incisal edges of anterior teeth.	Grade 0: no erosion. Grade 1: (Incipient); loss of surface features of the labial, lingual or occlusal enamel surfaces, giving a smooth, glazed appearance. The dentine is not involved. Grade 2: (Moderate): involvement of the dentine for less than one-third of the area of the tooth surface. Grade 3: (Grave): involvement of the dentine for more than one-third of the area of the tooth surface.

2.3.4 **Erosion Index and diagnosis according to** (Lussi, 1996).

Table 2.5 Erosion index and diagnosis according to (Lussi, 1996)

Surface	Score	Criteria
Facial	0	No erosion. Surface with a smooth, silky glazed appearance, possible absence of developmental ridges
	1	Loss of surface enamel. Intact enamel cervical to the erosive lesion; concavity on enamel where breadth clearly exceeds depth, thus distinguishing it from toothbrush abrasion. Undulating borders of the lesion are possible and dentine is not involved
	2	Involvement of dentine for less than half of tooth surface
	3	Involvement of dentine for more than half of tooth surface
Occlusal/ Lingual	0	No erosion. Surface with a smooth, silky glazed appearance, possible absence of developmental ridges
	1	Slight erosion, rounded cusps, edges of restorations rising above the level of adjacent tooth surface, grooves on occlusal aspects. Loss of surface enamel. Dentine is not involved
	2	Severe erosions, more pronounced signs than in grade 1. Dentine is involved

2.3.5 UK National Survey of Children's Dental Health Index (1999/2003)

Various epidemiological studies have used erosion indices based on O'Brien children's dental health in the United Kingdom1993 (Millward et al., 1994, Nunn et al., 2000, Dugmore and Rock, 2004a). O'Brien reported the use of the erosion index in children in the UK based upon the *1993 Survey of Children's Dental Health* that is based upon visual examination and the use of a CPITN probe which runs over the

tooth surface to check for loss of enamel surface characteristics. Selected teeth such as incisors and first permanent molars and selected surfaces such as buccal and lingual surfaces were examined on incisors and buccal, occlusal and lingual surfaces on molars (Table2.6). Codes were as follows:

Table 2.6 UK National Survey of Children's Dental Health index (1999/2003)

Depth	Area
0 Normal enamel 1 Loss of enamel surface characteristics 2 Loss of enamel exposing dentine 3 Loss of enamel and dentine with pulp exposure 4 Assessment could not be made	0 Normal 1 Less than one third of surface involved 2 Between one and two thirds of surface involved 3 More than two thirds of surface involved 4.Assessment could not be made

2.3.6 Using orthodontic study models (Ganss et al., 2001a)

Pre-orthodontic study models of 1000 children were examined of erosive lesions in primary and permanent dentitions. Moderate erosive lesions were found in 70.6% and advanced erosion in 26.4% of the children. Whereas, in the permanent dentition 11.6% of the 1000 children had at least one tooth with moderate erosion and 0.2% with advanced erosion. After a period of 5 years, 265 of the children were followed up by examination of their final study models. Subjects with erosive lesions in their deciduous dentition had a significantly increased risk (relative risk 3.9) for development of erosion in their permanent dentition. However for permanent teeth individuals with at least one tooth with moderate erosion had an increased risk from 5.3 to 23%; those with advanced erosion this risk was from 0.4 to 1.5%.

2.3.7 The Basic Erosive Wear Examination (BEWE)

A simple tool has been designed for use in general practice (Bartlett et al., 2008).

Table 2.7 The Basic Erosive Wear Examination (BEWE)

Criteria for grading	The calculation of BEWE scores
0 No erosion 1 Initial loss of surface texture 2 Distinct defect, hard tissue loss <50% of the surface area (dentine involved) 3 Hard tissue loss ≥50% of the surface area (dentine involved)	1. Sextant (17–14) 2. Sextant (13–23) 3. Sextant (24–27) 4. Sextant (37–34) 5. Sextant (33–43) 6. Sextant (44–47)

2.4 Mechanisms and pathogenesis of erosive tooth wear

The mechanisms of tooth wear fall into two distinct types: those of chemical origin (e.g. erosion) and those of physical origin (e.g. abrasion, attrition). In any individual, both chemical and physical insults to the tooth hard tissue will be present in some form or other, so tooth wear is the combined effect of these insults. Despite the clear definition of a number of distinct tooth wear mechanisms, it is uncommon to find a single wear mechanism (Pickles, 2006). The solubility of enamel powder increases dramatically with a decrease of pH (Larsen, 1990).

2.4.1 Chemical factors

The critical pH is the pH at which a solution is just saturated with respect to a particular mineral, such as tooth enamel (Dawes, 2003). Hydroxyapatite (HA), $Ca_{10}(PO_4)_6(OH)_2$ is the main component of the dental enamel, but it also contains several inorganic materials such as carbonate and fluoride (Dawes, 2003).

These inorganic materials vary among individuals, from tooth to tooth and within tooth surfaces that will also affect the solubility of the enamel (Weatherell et al., 1974, Dawes, 2003). When HA comes in contact with water (pH 7) some phosphate (PO_4), Ca^{2+} and hydroxyl ions will be slowly released into the solution until the water is saturated with respect to HA. At that equilibrium stage, where the rate of the forward reaction (mineral dissolution) is equal to the rate of the backward reaction (mineral precipitation) (Dawes, 2003).

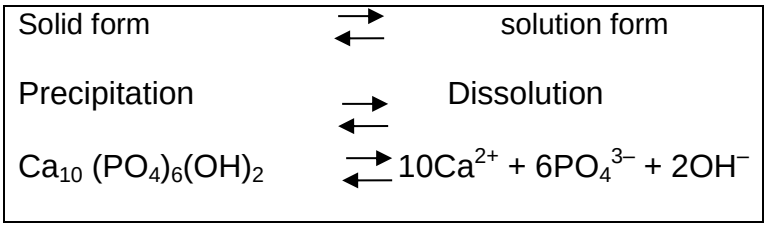


Figure 2.5 Enamel reactions in the solution

(Courtesy of Dawes, 2003)

Tooth mineral contains a calcium – deficient carbonated hydroxyapatite (Ca10-x Nax (PO_4)6-y (CO_3)z(OH)2-u F), which is different from stoichiometric hydroxyapatite $Ca_{10}(PO_4)_6(OH)_2$, with some of the calcium ions replaced by other ions such as sodium, magnesium or potassium (estimated to be approximately 1%). The enamel mineral is the calcium deficient of the teeth minerals is denoted by the 10_x after the Ca in the formula. Some of calcium ions may exchange with other metal ions, such as sodium, magnesium and potassium approximately 1% in total, with the sodium (Na) comprising the majority. In addition, some of the hydroxyl OH ions may also exchange with F, whereas the phosphate is designated as 6_y and the carbonate as z. However, if the substitutions in the crystal lattice occurs particularly in carbonate (CO_3) that replaces some of the phosphate (PO_4) but not on a one/one (stoichiometric) basis, subsequently will disturb and weaken the structure and render

the chemical structure of the mineral crystal lattice soluble (Featherstone and Lussi, 2006). Consequently, the tooth minerals become rather more soluble than hydroxyapatite which in turn is more soluble than Fluor apatite Ca_{10} $(PO_4)_6F_2$ (Featherstone and Lussi, 2006, Lussi et al., 2011).

The progression of acidic dissolution of the dental tissues is dependent on the type, pH, time of exposure of the acid and other mineral additives which cause ultra-structural changes. An in vitro study carried out on human and bovine enamel specimens which were treated with different acidic solutions and scanned by scanning electron microscopy showed that malic acid (pH 3.4) was less erosive than citric acid solutions at (pH 2.8) or phosphoric acid (pH 2.8) during immersion time at 15 mins, but the differences between the acidic solutions disappeared after further exposure of up to 60mins (Meurman and Frank, 1991, Meurman and ten Cate, 1996, Zero and Lussi, 2005).

Other factors play a crucial role in the erosive characteristics of the acidic solution such as titratable acidity (buffering capacity) and the chelating properties as well as the structure and composition of the enamel (Rytomaa et al., 1988, Zero and Lussi, 2005, Lussi and Jaeggi, 2006). The repeated exposure of the acidic solution will lead to histo-morphological change of the enamel either by dissolving the prismatic layer of the enamel by chemically wearing off the aprismatic layers, and with longer exposure to acidic attack the interprismatic areas will lead to a honeycomb appearance (Meurman and Frank, 1991, Meurman and ten Cate, 1996).

In vitro exposure to acidic drinks demonstrated alterations of the opening and permeability of dentinal tubules by removing the dentinal smear layer (Prati et al., 2003). However, the increase in the permeability and widening of dentinal tubules

as well as the erosion of the peritubular dentine by acidic solutions cause exposure of inner dentine structures to outer stimuli and lead to hypersensitivity of the dentine Meurman et al., 1991). Precipitation of phosphate-containing calcium fluoride crystals, $CaF_2(P)$, can cause severe reduction in the calcium ion concentration and release of hydrogen ions from the precipitated phosphate. These reactions result in considerable dissolution of enamel, HAP and even of FAP (Christoffersen et al., 1995).

$Ca_{10-x}\ Na_x\ (PO_4)6\text{-}y\ (CO3)z\ (OH)_{2-u}\ F_u + 3H^+ \rightarrow (10\text{-}x)Ca^{2+} + xNa^+ + (6\text{-}y)(HPO_4^{2-}) + z(HCO_3^-) + H_2O + uF^-$

Hydrogen ions, H_, result from acidic dissolution in water. For example, citric acid has the possibility of producing three hydrogen ions from each molecule; The H_ ion can attack the tooth mineral crystals and directly dissolve by combining with either carbonate or phosphate ions, as shown in the equation leading to direct surface etching.

Citric acid exists in water as a mixture of hydrogen ions, acid anions (e.g. citrate) and attached acid molecules, with the amounts of each determined by the acid-dissociation constant and the pH of the solution. The hydrogen ion directly attacks the crystal surface. Over and above the effect of the hydrogen ion, the citrate anion may complex with calcium, also removing it from the crystal surface. Each acid anion has a different strength of calcium complexion dependent on the structure of the molecule and how easily it can attract the calcium ion. Thus, acids such as citric acid have double actions. Hydrochloric acid dissolves completely in water to hydrogen ions and chloride ions, rapidly and directly dissolves and removes the mineral surface. The chloride ion plays no role in the demineralisation process (Lussi and Jaeggi, 2008).

2.4.2 The multifactorial processes influence in dental erosion

The diagram proposed by (Lussi, 2009), showing the multifactorial processes that interplay in the mechanism of erosion is shown in Figure 2.6.

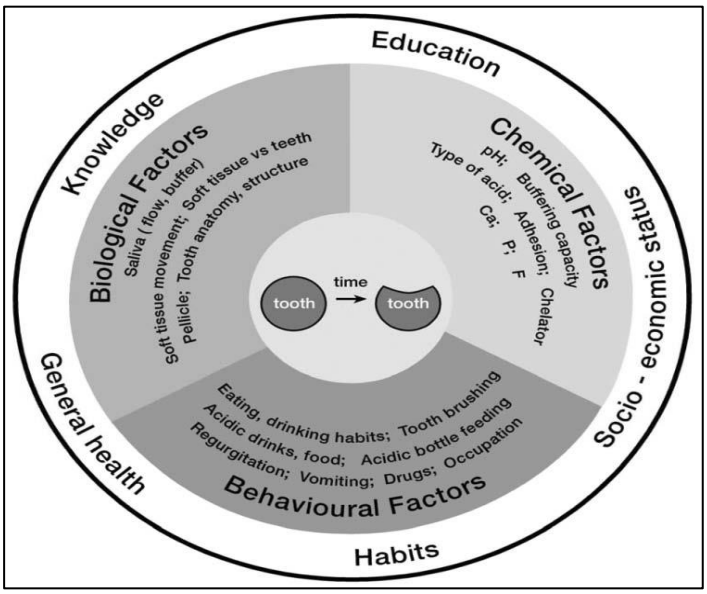


Figure 2.6 Interactions between the protective and risk factors for the development of erosive tooth wear as suggested by (Lussi 2009)

Dental erosion (erosive dental wear) is a cyclic process influenced by mineral loss due to acidic consumption, remineralisation and mechanical action of the toothbrush and the dental tissues exposed to such conditions for longer periods of time, combined with the use of highly abrasive dentifrices can lead to greater tooth loss (Hara et al., 2009b). Interplay by multiple factors such as chemical (pK values, adhesion and chelating properties, calcium, phosphate and fluoride contents), behavioural (eating and drinking habits, life style and excessive consumption of acids) and biological (salivary flow rate and buffering capacity) play a role in the development of dental surface wear (Zero and Lussi, 2005, Lussi et al., 2006, Lussi, 2009). Low socio-economic status and frequent consumption of carbonated and fruit drinks are related to the severity of erosive tooth wear (Al-Dlaigan et al., 2002, Harding et al., 2003).

Erosive tooth wear is the physical result of a pathological, chronic, localised loss of dental hard tissues that is chemically etched away from the tooth surface by acid and /or chelation without bacterial involvement (Imfeld, 1996b). The different risk and protective factors and their interaction should be considered for the comprehensive preventive approach for the management of dental erosion (Lussi, 2009).

2.4.3 Other influencing biological modifying factors

The variations of the anatomical physiological characteristics of the biological oral environments affecting the erosive tooth process include tooth composition and structure, oral soft tissues, swallowing patterns, salivary fluid and pellicle formation (Zero and Lussi, 2005). The pellicle which forms shortly after 3 mins has been shown to provide an erosive protection against citric acid demineralisation (Hannig et al., 2004). The natural salivary protection role in erosive process includes dilution and clearance of erosive acid attack in the mouth, neutralisation and buffering of acids, providing some minerals such as calcium, phosphate and possibly fluoride to the dental hard tissues to maintain the supersaturating phase necessary for the remineralisation process (Zero and Lussi, 2005). Of the many salivary parameters that have been considered, only the unstimulated salivary flow rate and buffering capacity have been directly associated with dental erosion (O'Sullivan and Curzon, 1999). Any procedure that removes or reduces the thickness of the pellicle may compromise its protective properties and accelerate the erosion process. Procedures such as tooth brushing with abrasive toothpaste will remove the pellicle (Zero and Lussi, 2005, Ganss et al., 2011b).

Hence, the chemical processes that cause erosion are complex. Any fluid that comes in contact with the dental enamel surface has to penetrate through the

salivary pellicles, which is an organic biofilm resulting from salivary glycoproteins which cover and protect the surface of teeth. A developing young pellicle will hardly be a diffusion barrier to an erosive agent. Only when the pellicle has matured and has achieved a certain thickness can it slow down the diffusion process. Once in contact with enamel, the acid with its hydrogen ion (or with its chelating capacity) will start to dissolve the crystal (Hannig and Balz, 1999, Amaechi et al., 1999b).

The un-ionised form of the acid will then diffuse into the interprismatic areas of enamel and dissolve mineral in the subsurface region. This will lead to an outflow of tooth mineral ions (calcium and phosphate) and subsequently to a local pH rise in the tooth structure in close proximity to the enamel surface. This process is stopped when no new acids and/or chelating substances are provided. An increase in agitation (e.g. when a patient is swishing a drink in the mouth) will enhance the dissolution process, because the solution on the surface layer adjacent to enamel will be readily renewed. Furthermore, the amount of drink in the mouth in relation to the amount of saliva present will modify the dissolution process. Citric acid commonly present in many soft drinks may act as a chelator capable of binding minerals (calcium) of enamel or dentine, thus increasing the degree of below saturation and favouring more demineralisation (Zero and Lussi, 2005). However, salivary pellicles are able to protect against short erosive attacks (Nekrashevych and Stösser, 2003) and according to the thickness and the site specificity (Amaechi et al., 1999b).

2.5 Epidemiology

The existence of dental wear was demonstrated even in medieval skulls and past historic era (Ganss et al., 2002, Caglar et al., 2007, Esclassan et al., 2009). Epidemiological studies showed that the global increase in interest and the prevalence of dental wear and in particular more attention in erosive tooth wear among different populations in various countries (Kelleher and Bishop, 1999, Hefferren, 2004).

A systematic review was performed by (Kreulen et al., 2010) found that the prevalence of dental wear exposing dentine ranged from 0 to 82% in primary teeth of children up to 6.5 years in 13 studies and from 0 to 54% in permanent teeth of children 7 years and older in 18 studies. Furthermore, dentinal involvement of primary teeth linearly increased with the age of the children and 17 of the reviewed studies reported there was a relationship between dental wear and gender (Kreulen et al., 2010).

Enamel loss due to acidic erosion or a combination of erosion and abrasion is highly prevalent in modern countries and has a high incidence rate among teenagers from 56.3% to 64.1% in a group of children aged 12 and 14 years (Dugmore and Rock, 2003). A longitudinal study over a 1.5 years period on a group of adolescents found that 24.2% of erosion-free children developed erosion over 1.5 years and deep enamel erosion increased from 1.8% to 10.8% and 2.6% showed dentine exposure during the study period (El Aidi et al., 2008). There were 28% of the children that had dental erosion in a total sample of 153 healthy, 11-year-old school children that were sampled from a downtown public school in Istanbul, Turkey (Table 2.8)

Table 2.8 Summary of different epidemiological studies of prevalence percentages of dental wear in different populations (in order by year of publication)

Country	Age (years)	Sample size	prevalence	authorship
Saudia	19 – 25	90	38 individuals with dental wear	(Johansson et al., 1991)
UK	4	178	50%	(Millward et al., 1994)
UK	3	135	28.9%	(Jones and Nunn, 1995)
UK	5	>1000	24	(Downer, 1995b)
Saudia	19 – 25	95	28% of maxillary anterior	(Johansson et al., 1996)
UK	11-13	125	37%	(Deery et al., 2000)
USA	11-13	129	41%	(Deery et al., 2000)
Cuba	12	1010	17.4%	Künzel, Cruz and Fischer 2000)
UK	14	418	48% low erosion 51% moderate erosion	(Al-Dlaigan et al., 2001a)
Saudia	5 - 6 12–14	354 boys 862 boys	34% 26% In dentine	(Al-Majed et al., 2002)
Ireland	5	202	21	(Harding et al., 2003)
UK	12	1,753	59.7%	(Dugmore and Rock, 2004b)
UK	14	2,351	53% exposed dentine	(Bardsley et al., 2004)
Istanbul (Turkey)	11	153	28%	(Caglar et al., 2005)
China	3 - 5	1949	5.7%	(Luo et al., 2005)
Australia	5.5 -14.6	714	(68%)	(Kazoullis et al., 2007)
Ireland	16 - 24	1191	38.1%	(Whelton, 2007)
Ireland	35 - 44	959	76.2	(Whelton, 2007)
Ireland	65+	406	93.0%	(Whelton, 2007)
Sweden	5 - 6	153	13.3%	(Hasselkvist et al., 2010)
Sweden	13 -14	227	11.9 %	(Hasselkvist et al., 2010)
Sweden	18 -19	247	22.3 %	(Hasselkvist et al., 2010)
Libya	12	791	40.8%	(Huew et al., 2012)

2.5.1 Erosive wear in children

There is a trend towards an increase in erosive dental wear in children (Nunn et al., 2003, Linnett and Seow, 2001). In 1993 an important report from the national survey was carried out in children in the UK highlighted the high prevalence of dental erosion among children encouraged to provide proper dental care (Downer, 1995a).

A high prevalence of erosion in children aged between 3½ and 4½ years and in those who consumed carbonated drinks on most days had more buccal and palatal erosion compared with toddlers consuming these drinks less habitually. Moreover drinking overnight was associated with an increased prevalence of erosion as was medical history symptoms of gastro-oesophageal reflux in 4–6-year-olds who exhibited erosion compared with symptom-free children (Nunn et al., 2003).

Dental examinations on 714 children aged 5.5 to 14.6 years from 8 randomly selected Australian schools revealed that 225 children (32%) who presented without erosion and 489 (68%) who had erosion of at least one tooth. Moreover, the prevalence of dental erosion in the primary dentition was 3 times (78%) greater than for the permanent dentation (25%) (Kazoullis et al., 2007). In the study of the dental health of 3-year-old children in East Cumbria of 135 children, 39 (28.9%) had one or more maxillary incisors affected by erosion (Jones and Nunn, 1995).

Nearly half of the assessed 178 four-year-old children showed signs of erosion. The palatal surfaces of the upper incisors was the most common site affected comprising 17% of the children examined showing visible dentine for greater than one third of the tooth surface (Millward et al., 1994). The evidence of dental erosion in a sample of 202 of 5 year old Irish children was 47% and 21% into dentine. The associated variables for dental erosion both in fluoridated and non-fluoridated areas were similar, while, involvement of dentine or pulp was associated with low socio-economic status and frequent consumption of carbonated and fruit drinks (Harding et al., 2003).

Children who suffered erosive lesions in their primary dentition had a significant risk to develop erosion in their permanent teeth ($P \leq 0.001$) (Ganss et al., 2001).

2.5.2 Erosive wear in adolescence

A follow-up random sample of 1,753 children was examined at age 12 years and 1,308 of the same children were re-examined at age 14 years, 645 (56.1%) had erosion at 12 – years-old and after 2 years later the estimation was 750 (65.3%) (Dugmore and Rock, 2004a, Dugmore and Rock, 2004b). The estimated measurements of erosion of the upper permanent incisors among 125 UK subjects that comprised of 51 males and 74 females and 129 USA subjects comprised of 58 males and 71 females of 11-13 years olds were 37% and 41% respectively (Deery et al., 2000). The estimation of erosion experience In 12 years old Libyan school children was 40.8% (Huew et al., 2012).

2.5.3 Erosive wear in adults

During the adult dental health survey in 1998 in the UK it was reported that 11% of adults had tooth wear on anterior teeth which was rated as moderate or worse (Nunn et al., 2000). Seventy-seven per cent of 1010 university students had at least one tooth with tooth surface loss into dentine. Overall OHIP (Oral Health Impact Profile) scores were similar for individuals with different levels of severity of tooth surface loss. Individuals with severe tooth surface loss were more likely to report that their appearance had been affected by, and that they had felt self-conscious because of the condition of their mouth and teeth (Daly et al., 2011). An increase in tooth wear with age was observed in a sample of 586 dentate adults aged 45 years and the most affected surfaces were occlusal, incisal and cervical areas (Donachie and Walls, 1995).

2.6 Gender predilection

Some epidemiological studies demonstrated that dental wear was more prevalent in males than females (Seligman et al., 1988, Dahl et al., 1989, Donachie and Walls, 1995, Kelly et al., 1998, Al-Dlaigan et al., 2001b, Árnadóttir et al., 2003, Bardsley et al., 2004). However some studies showed girls had more erosive teeth than boys in an area close to orange plantations (Künzel et al., 2000).

2.7 Aetiology

The aetiology of dental erosion can be classified into extrinsic or intrinsic acid or a combination (Amaechi and Higham, 2005, Imfeld, 1996b), among the extrinsic sources include fruit juices and acidic beverages, acidic medications, occupational, life style and environmental. Intrinsic erosion is caused mainly by gastric acids.

The leading factor in tooth wear is the interaction between erosion of dental hard tissues by either exogenous factors such as dietary and drinks (Duggal et al., 1995, Tahmassebi et al., 2006) or endogenous acidic sources and intra-oral abrasive forces, such as those caused by tooth brushing (Imfeld, 2001).

2.7.1 Extrinsic aetiological factors

2.7.1.1 Diet

Dietary acids are one of the most important extrinsic factor in initiation of erosive wear of the teeth and among those are the citrus fruits such as lemon, orange and moreover acidic fruit - flavoured candies and snacks in which the contained acid-related fruit flavours such as lemon, cherry, and grape that may destroy much more enamel than the other neutral aromatic flavours, such as cinnamon and mint (Bibby

and Mundorff, 1975). There is a relationship between the presence of dental erosion in children and intakes of fruit drinks from feeding bottles or consumption of fruit drinks at bed time (Luo et al., 2005). An *in situ* study showed that primary teeth were more liable to erosive wear than permanent teeth in the oral environment (Hunter et al., 2000).

Marked dental erosion at the mesial edges of upper front teeth was observed during an examination of Cuban children that presented with the characteristic V-shaped defects on upper central incisors which was related to the manner in which citrus fruits were eaten. There was also a positive correlation between the frequency of dental erosion near the region of citrus plantations, which was probably related to the extent of (daily) orange eating (Künzel et al., 2000).

Interesting findings were found in 63% of the subjects who consumed acidic diets demonstrated as shallow defects on buccal surfaces, localised coronal from the enamel-cementum junction (Ganss et al., 2002).

2.7.1.2 Drinks

The consumption of soft drinks and fruit juices such as apple and orange drinks were more prevalent among young children and school teenagers (Dennison, 1996, Lytle et al., 2000). It was estimated that 80% of 14 year old school children regularly consumed soft drinks. Also the report showed that 13% and 10% respectively had more than 22 intakes per week of cola and other carbonated drinks, in addition to this almost a quarter of these 14-year-olds had alcoholic drinks, 34% sport drinks and a small minority of the sample (3%) consumed beer and cider between 8 and over 21 times per week; 21% and 15% respectively drank some wine or spirits (Al-Dlaigan et al., 2001b).

A longitudinal assessment of a follow-up of 265 children from 1000 individuals using their final orthodontic study models over 5 years, found that in their primary teeth, 26.4% of the individuals had no erosive lesions, whereas, grade 1 erosion was present in 70.6% of them and grade 2 erosion was found in 26.4%. The occlusal/incisal surfaces of primary teeth were the commonly affecting sites compared to the oral or vestibular surfaces Grade 1 erosion was found in 44% of the occlusal surfaces of molars (36% of the incisal surfaces of the canines), and grade 2 erosion in 11% (9%). In the permanent teeth, 11.6% of individuals had at least one tooth with grade 1 erosion but only 0.2% had at least one tooth with grade 2 erosion (Ganss et al., 2001b). The author attributed the increase in the erosive lesions and the remarkable significant increase of the intake of fruit and acidic beverages per capita during the last two decades in Germany. The results of tooth wear in 210 schoolchildren in London in the summer of 1996 were 57% of children had tooth wear on more than 10 teeth and a median 12% of surfaces were affected while dentine was rarely affected and an average of 2 cans intake of carbonated drinks per day. It was also found that there was no relationship between salivary flow rate or buffering capacity and those who had a history of regurgitation demonstrated a higher maxillary TWI compared to others (Bartlett et al., 1998).

History of consumption of certain erosive drinks such as orange juice, carbonated beverages, fruit yogurt were reported in children who suffered dental erosion 32%, 40% and 36% respectively in a total sample 135 school children (Caglar et al., 2005). Positive associations were found between drinking fruit juice or fizzy pop and with erosion and caries involvement (Dugmore and Rock, 2004b).

2.7.1.3 Sports

Swimmers are prone to dental erosion due to improperly chlorinated swimming pools. A case report of a home swimming pool showed an association of erosion with severe sensitivity of teeth with dark staining and rapid loss of enamel which was due to an improperly chlorinated water pool (Jahangiri et al., 2011). More than half (60%) of 28% of children who suffered dental erosion swam professionally in swimming pools (Caglar et al., 2005). Acid erosion of dental enamel or “swimmer’s erosion” is commonly seen among competitive swimmers which can be caused by inadequately maintained gas-chlorinated swimming pools. An epidemiological survey was conducted of 747 club members, and 39% of the swimming team members presented with symptoms compatible with dental enamel erosion whereas 3% of non-swimmers, 12% of swimmers who were not members of the swimming team (Centerwall et al., 1986).

Dental erosion was identified in 25.4%of athletes in 32 sports clubs (690 members) of the University of Melbourne who participated in a survey and frequent association with fruit juice drinks was shown (Sirimaharaj et al., 2002).

2.7.1.4 Environmental and occupational erosive wear

Industrial or environmental dental erosion was found to be prevalent among industrial workers such as in battery formation, galvanising and associated workers (Wiegand and Attin, 2007). Environmental erosion especially affects the maxillary and mandibular anterior teeth due to an exposure to inorganic acid fumes from the work environment which may increase the erosion of these teeth, which are not continuously protected by saliva and the lips (Kelleher and Bishop, 1999). Approximately 176 (3I.7%) among 555 acid workers examined between March I962

and October I964 were affected by industrial dental erosion and 33 cases had erosion in dentine (ten Cate, 1968). The prevalence of erosion was 31% among German battery factory workers (Petersen and Gormsen, 1991). There was a relationship between presence of zinc and dental erosion in 7 out of 12 Norwegian industry workers and also the degree of erosion was related to the length of service in years (Skogedal et al., 1977), as was also reported among the 157 workers that participated from four acidic factories of which, 76 were working in departments containing acid fumes, and 81 had never worked under such conditions and were used as the control group. Of the acid workers 18.4% had one or more teeth with erosion, and was 8.6% for the control group (Tuominen et al., 1989). Wine erosion is recognised as another occupational hazard among wine industry tasters (Piekarz et al., 2008), in a case report of wide spread dental erosion that was documented in a person who worked as a wine market taster (Gray et al., 1998).

2.7.1.5 Life style

Modern life styles have changed for both food consumption and eating habits among different populations (ten Cate and Imfeld, 1996). It is a common finding to consume more acidic foods and drinks prior to examinations among school teenagers and in the same study erosion was noticed in 21.6 % of a sample of 278 of 15 year children (Árnadóttir et al., 2003).

2.7.1.6 Socio - demographic.

Specific socio-demographic regions showed the strongest association with erosion. It was observed that the residential area showed the strongest relation with prevalence of erosion, as noticed in those young people who lived in the North of England and had double the erosion level compared with those living in London and

the South-East. In the youngest age group, the most significant association was with family house type that is, living with both parents and a number of siblings compared with, for example, as an only child or with a single parent in receipt of benefit (Al-Dlaigan et al., 2001a). For adolescents, the associations that were strongest were again geographical, north versus south, social class of the head of household, manual versus non-manual occupation, and age, older versus younger adolescents (Al-Dlaigan et al., 2002, Al-Malik et al., 2002, Nunn et al., 2003) .

Climatic and geographic locations e.g. a harsh desert may be responsible for major passive abrasive aetiological factors in a sample of Saudi individuals (Johansson et al., 1991). Children who lived in non-fluoridated areas tended to have more smooth dental wear than those who lived in fluoridated areas (Bardsley et al., 2004).

2.7.2 Intrinsic erosive wear

2.7.2.1 Chronic diseases and acidic medication

The manifestation of dental erosion due to chronic underlying medial diseases or various acidic oral medicine liquids and effervescent preparations routinely prescribed for long term use by paediatric renal patients can be observed during regular persistent acidic exposures on the structure of the teeth only in those diseases which were associated with chronic vomiting or persistent gastroesophageal reflux over a long period. Examples of such conditions include disorders of the upper alimentary tract, specific metabolic and endocrine disorders, cases of drug side-effects and drug abuse, and certain psychosomatic disorders, e.g. stress-induced psychosomatic vomiting, anorexia and bulimia nervosa or rumination

(Scheutzel, 1996, Nunn et al., 2001).

Antihistamine - containing syrup showed an *in vitro* reduction of the primary enamel hardness (Costa et al., 2006).

2.7.2.2 Asthma

With a worldwide increase in asthma sufferers (Anderson, 2005, Asher et al., 2006), a tooth structural loss was reported due to the brushing of teeth immediately after the use of dry powder inhalers (Manuel et al., 2008).

A high level of erosive tooth wear was found in a group of asthmatic individuals compared to a non-asthmatic control group and history of dental hypersensitivity, xerostomia, abnormities of salivary gland and self – induced vomiting. Moreover, 60% had suffered gastro-oesophageal reflux (GOR) among asthmatic patients (Sivasitamparam et al., 2002). The prevalence of asthma in a random sample of 418 fourteen-year-old children in Birmingham UK was 15.8% (66 children out of 418) and the levels of dental erosion in children with asthma were higher (Shaw et al., 2000).

2.7.2.3 Alcoholism

A higher risk of developing dental erosion was found in a group of clinically diagnosed alcoholic participants compared with a control group who did not consume alcohol and might be due to the reduced pH of both the stimulated and unstimulated saliva in the alcoholic group. Gross erosive dental wear was reported in cases with long term alcohol abuse (Smith and Robb, 1989, Dukić et al., 2010).

2.7.2.4 Eating disorders

The average prevalence rates for anorexia nervosa and bulimia nervosa among young females are 0.3 and 1%, respectively (Hoek, 2006). It is believed for the

development of such diseases among certain professional subculture groups e.g. (dancers and models) where dieting and a demand for thinness are common (Garner and Garfinkel, 1980). Bulimia is more common than anorexia with an incidence of between 8.6 and 14 per 100,000. In general, self-induced vomiting resulted in an increased frequency of erosion on palatal surfaces but both research groups noted that the diet of their subjects included significant quantities of low beverages and fresh fruit. The latter was eaten to induce diarrhoea (Milosevic et al., 1997, Milosevic, 1999).

2.7.2.5 Gastro – oesophageal reflex disease (GORD)

Chronic regurgitation is the reflux of gastric juice through the upper oesophageal sphincter and comes in contact into the oral cavity due to failure of the anti-reflux mechanism, which is predominantly controlled by the lower oesophageal sphincter which may cause potential damage to the teeth (Bartlett et al., 2007, Hershcovici et al., 2011).

Insidious vomiting and acid regurgitation history was found in 21 out of 90 Saudi adults (Johansson et al., 1991), although 79% of the 4–6-year-olds that had reported symptoms related to gastro-oesophageal reflux had dental erosion compared with 62% who did not have such symptoms.

For a UK study, 53 of the examined children with moderate to severe GORD fulfilled the study entry criteria, 28 from Leeds and 25 from group of children from London ranging in age from 2 to 16 years, evidence of erosion was seen in 9 (17%) out of 53 children, on the palatal surfaces of the upper primary incisors (O'Sullivan et al., 1998).

2.8 Methods to monitor tooth surface loss

Various quantitative and qualitative techniques have been used to assess changes in dental tissues (Attin, 2006).

2.8.1 Quantitative methods

They include atomic absorption spectroscopy (Hannig et al., 2004), microhardness tests and iodide permeability (Lussi et al., 1993), microradiography (Amaechi et al., 1999a), surface profilometry (Eisenburger et al., 2000), confocal laser scanning microscopy (CLSM) and quantitative light-induced fluorescence (QLF) (Sano et al., 2007), ultrasonic measurement of enamel thickness (Huysmans and Thijssen, 2000), atomic force microscopy and nano-indentations (Barbour and Rees, 2004).

2.8.1.1 Microhardness tests

Microhardness testing measures the resistance of enamel surfaces to indenter penetration by using either a Knoop or a Vickers diamond indenter, which are rhomboidal and tetra-pyramidal, respectively and is used mainly to assess the degree of the superficial enamel layer porosity that shows mineral loss or gain in subsurface lesions (Koulourides, 1971). In this method, the selected indenter type with a well-defined load and time should be placed on the sample to obtain an indentation in the tooth surface and the indentation length is then calculated microscopically (in µm). The Knoop diamond produces a diamond-shaped indentation whereas the Vickers diamond produces a rectangular-shaped indentation (ten Bosch and Angmar-Månsson, 1991).

The Knoop diamond indenter penetrates sound enamel by about 1.5 µm , while that of Vickers would penetrate about 5 µm given the usual loads of 50 and 200 g,

respectively (Featherstone, 1992), as acid attack will deepen the indentation (Attin, 2006). Knoop indenters are to be preferred because they have a long and a short axis, whereas Vickers indenters are symmetrical. Therefore, Knoop hardness is thought to be more sensitive to changes in the most superficial layer of an erosive lesion. Changes in surface hardness of enamel can be observed even after a few minutes of exposure to an erosive agent (Hara and Zero, 2008). The disadvantage of this method is that in cases of more acid exposure of eroded dental substrates the indentation margins are not clearly defined, so that measurements are either inaccurate or impossible since erosion affects both indented and non-intended surfaces after erosion (Attin, 2006, Shellis et al., 2011). Thus, the reduction in in the surface of advanced erosive tissues cannot be measured clearly by hardness measurements of the remaining surface. Another limitation is that, when material is deposited on the surface, e.g. by application of certain fluorides, surface hardness measurements may not be representative (Schlueter et al., 2011). Both SMH and Iodide permeability tests can be utilised to detect early changes of early enamel demineralisation (Zero et al., 1990).

For the most accurate assessment of enamel hardness, flattened polished surfaces are necessary and the test surface must be positioned perpendicularly to the long axis of the indenter. These requirements limit the accuracy of hardness measurements on natural tooth surfaces (Schlueter et al., 2011).

There are two types of microhardness testing, i.e. surface microhardness and cross-sectional microhardness. Surface microhardness (SMH): in which a load with a diamond indenter is applied perpendicular to a polished dental surface. SMH is a non-destructive technique that allows for a longitudinal study of the same specimen, however it cannot provide details about the subsurface hardness changes or inform

about any structural alterations to different sides of the lesion (Featherstone et al., 1983). Cross-sectional microhardness (CSMH): where the diamond indenter load is applied parallel to the tissue‘s anatomical surface (Souza et al., 2013). CSMH experiments include the ability to provide indirect evidence of mineral loss or gain as well as the possibility to obtain the mineral profile (volume percentage of mineral as a function of the distance from the outer surface). However, CSMH experiments cannot include the outermost 25 µm of a sample in the measurement (Arends & ten Bosch, 1992).

2.8.1.2 Nano-indentation

It uses the same principle as microhardness indentation but at a smaller scale. It uses a special diamond indenter that produces an indentation usually maximally 1 µ m in length under loads of 0.25–50 mN (Mahoney et al., 2003).

Because of their deeper penetration, micro-indenters are thought to be influenced not only by the displaced area but also by its surroundings, which may involve sound enamel in shallow lesions. With nano-indentation, there is the likelihood of detecting the Young modulus (elastic deformation). This seems a useful parameter for the representation of very shallow erosive lesions, considering that it would not be influenced by the underlying intact enamel (Barbour and Rees, 2004, Attin, 2006, Schlueter et al., 2011). Thus, the use of elastic modulus is sometimes considered a better parameter to assess the effect of an acid impact (Barbour and Rees, 2004). Flattened and polished surfaces are required for adequate measurements, but it has been suggested that, because of the small extent of the measurement, the natural curvature of the tooth may not be a problem for nano-indentation (Attin, 2006).

Nano-indentation of dentine is frequently used with atomic force microscopy (AFM) (Lippert et al., 2004, Attin, 2006).

2.8.1.3 Microradiography

Microradiography is a tool for quantification of mineral loss based on the attenuation of X-ray irradiation transmitting through a dental hard tissue by comparison with a reference aluminium step wedge. Transmitting a dental hard tissue sample can be recorded by photo-counting X- ray detectors, or X-ray sensitive photographic plates or film. The mineral mass can be calculated from the photon counts or values of photographic plates or film knowing the appropriate mass attenuation coefficient or by determining photographic density measurements calibrated by an aluminium step-wedge and the images can be analysed by controlled computer software and mineral loss should obtained by plotting the volume % mineral profile towards dentine depth in each dentine section with the sound dentine set as 48 volume mineral. Lesion depth was defined as the distance from the surface to the site of which mineral content was more than 95% of the sound dentine. Loss of dentine surface was considered as the distance from the virtual surface (defined by the non-demineralised surface) to the site in which mineral content started to be detected (Hara et al., 2005), assessment of photographic plates or film densitometers or, more recently, CCD cameras attached to a microscope are in use (Attin, 2006, Schlueter et al., 2011). The main advantage of microradiography is that the method enables simultaneously determination of surface loss of the eroded samples (Amaechi and Higham, 2001). In transversal microradiography (TMR), the X-ray beam is perpendicular to the direction of the experimental sample while in longitudinal microradiography (LMR) the beam is approximately parallel with this (de Jong et al., 1987a, De Jong et al., 1987b). These approaches use X-rays at a

specific wavelength. Hall et al. (1997) found a strong correlation between mineral loss determined by either TMR or profilometry even for early erosive lesions caused by erosion exposures of less than 1h. Another approach to use TMR for erosive mineral loss determination also depends on the use of reference areas not subjected to an erosive challenge (Amaechi et al., 1998a). TMR was used to record lesion depths from 20 µm and more. For determination of mineral changes following a small erosive challenge, e.g. erosive surface softening only, this technique is not sensitive enough due to the fuzziness of the outer 5–10 µm at the edge of the dental hard tissue slabs prepared for TMR. Longitudinal microradiography (LMR) enables the use of thicker specimens up to 4mm thickness usually cut from the tooth comprising the natural enamel surface and some underlying dentine. However, use of thinner specimens provides better information about the mineral change within the specimen. The specimens are radiographed perpendicular to the surface before and after treatment(s), and changes in mineral content can be calculated using pixel by pixel comparison of the radiographic readings after treatment with the values of the reference radiograph (De Jong et al., 1987b, Ganss et al., 2004b, Ganss et al., 2005). In contrast to TMR, LMR is not able to determine the mineral profile of a specimen from the surface to depth. Since LMR enables the reuse of specimens, it can be used for longitudinal observations. The mineral loss recorded with LMR consists of both the erosive crater and the loss of mineral in the softened surface zone. LMR is less sensitive to minute changes in mineral content than TMR, because of the use of thicker specimens as compared with TMR. Using LMR, erosion progression in both enamel and dentin has also been assessed (Ganss et al., 2001a, Ganss et al., 2004b, Attin, 2006). In these studies, the method has shown to be suitable to allow for distinction of different preventive treatment modalities

resulting in different mineral loss. The comparison of LMR in enamel specimens with either profilometry or analysis of dissolved calcium/phosphorus showed good correlation for the three methods (Ganss et al., 2005). However, it also became clear that losses below 20m should be interpreted with care when using LMR only, since standard deviations were quite high when determining minimal substance loss with LMR (Ganss et al., 2005).

2.8.1.4 Confocal laser scanning microscopy (CLSM)

Uses monochromatic laser light to collect images from specific focal planes. Images from a series of focal planes can be combined by computer software to generate 2D optical sections perpendicular to the focal plane or 3D images. The results of mineral content and morphological changes due to demineralisation can be drawn from the alterations in reflection and scattering of light (Zentner and Duschner, 1996, Schlueter et al., 2011). The advantages of CLSM are the high resolution (less than 300 nm in the x and y directions and 20 nm in the z direction) and fast recording of the surface topography. CLSM is mostly used to obtain qualitative information, but it also can be tested for the erosive potential by immersing each enamel specimen (10 per group) into solutions of the various products for 10 and 20 min. Before and after the experiment Knoop surface hardness (SMH) was measured. The enamel microstructure before and after immersion was assessed by scanning electron microscopy and confocal laser scanning microscopy and the change of microstructure of polished enamel before and after immersion was assessed with confocal laser scanning microscopy (CLSM). There were correlation with the results of the measurements of enamel surface loss by the surface hardness, the SEM and the CLSM (Lussi and Hellwig, 2001).

2.8.2 Qualitative methods

For example, scanning electron microscopy (SEM) (Barbour and Rees, 2004), environmental scanning electron microscopy (Attin, 2006).

2.8.2.1 Scanning electron microscopy (SEM)

SEM can be utilised to study ultrastructural changes associated with erosion in both enamel and dentine (Sorvari et al., 1996). In the case of enamel, surface etching and exposure of enamel prisms may be the results of an acid attack due to specimen immersion in erosive solutions (Meurman and Frank, 1991). In dentine, exposure to acid challenges may create an opening of dental tubules (Meurman et al., 1991).

The main advantage of environmental SEM (ESEM) is that there is no need to create sample preparation and also it allows examination of samples in wet conditions without metal or carbon coating. When SEM combined with energy - dispersive X-ray spectroscopy could provide information about the composition of a specimen from the characteristic X-rays released under electron occurrence. Energy-dispersive X-ray spectroscopy can thus be used to determine quantitative changes in elemental composition on both eroded surfaces and cross sections. It can also be applied to detect the deposition of active agents from therapeutic treatments at the tooth surface and underneath the surface from concentration profiles of the piece in cross sections (Ganss et al., 2010, Schlueter et al., 2009b, Wiegand et al., 2009b, Charig et al., 2004).

2.8.3 Other chemical methods

Include chemical analysis of dissolved minerals (calcium & phosphate) in the erosive solution (Attin, 2006). The disadvantages of this method are that the presence of

saliva during the erosive challenge could cause interference with the analysis. Furthermore, this method cannot give information about possible mineral gain, or about any physical and morphological changes of dental hard tissues (Schlueter et al., 2011).

2.8.4 Non – contact optical surface profilometry (Proscan 2000, UK) [1]

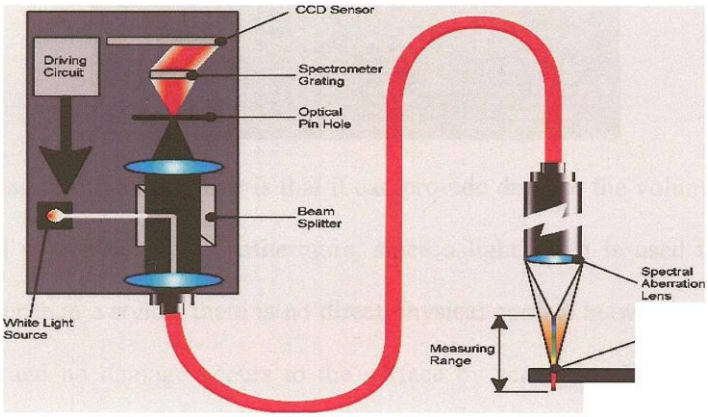


Figure 2.7 Non - contact optical profilometry (Proscan 2000) UK

Courtesy of (Abdullah et al., 2009)

Profilometry is a method of recording the amount of surface loss (µm) of dental hard tissues. It has a diamond stylus of a tip radius of 20 µm and a recording velocity of 10 mm/min. The force applied by the stylus to the samples varied linearly with deflection at a rate of 8 mg/µm to a maximum of 1 g at 100 µm. The profilometer software calculated the mean level relative to two reference points (Eisenburger and Addy, 2002, Eisenburger et al., 2003).

In this technique, the enamel surface is divided into two parts, an exposed part which is the experimental area and an unexposed dental surface which acts as a reference. The reference areas may be protected using two coats of nail varnish or tape. The

[1] http://www.scantronltd.co.uk/products-and-systems/--proscan-2000/17/

sample surface is scanned before and after erosion, and the amount of material loss can be measured from the trace produced. The non-contact profilometer has been used to assess tooth surface loss in various i*n vitro* and *in situ* studies, in which technique using the traditional contact stylus is replaced with white light or a laser, and interferometry is used to build up a map of the surface. Light profilometry can provide data for the volume of enamel loss as well as the vertical loss which is considered as one of its main advantages (Barbour and Rees, 2004, Attin, 2006).

In addition, there is no damage to the enamel surface since there is no direct physical contact between the probe and the surface. Furthermore, the specimen size can be varied from a small enamel sample of 1mm to an entire study model because of the interchangeable scanning heads available.

Profilometry has many advantages over other methods such as being a fast and simple technique that can be used over a relatively large area of enamel and does not cause any scratches or damage to the eroded area. However, the disadvantage of this technique is that the enamel sample has to be ground flat before scanning. Furthermore, this technique is used for the more advanced stages of erosion than indentation techniques as it measures surface loss rather than surface softening (Barbour and Rees, 2004).

2.9 Prevention

It is essential to establish a preventive program for the patients suffering from erosive tooth wear based on patho-physiological conditions in order to (1) reduce the frequency and severity of acid challenge, (2) improve the salivary flow rate, (3) to increase acid resistance, remineralisation and rehardening by fluoride application, (4)

to offer chemical protection by buffering substances, (5) to lessen abrasive tooth wear and (6) provide mechanical protection (Imfeld, 1996b). Early identification, examination and diagnosis are much important in the management of tooth surface loss (O'Sullivan and Milosevic, 2008).

2.9.1 Identification of the possible causes of erosive tooth wear

As recommended by The UK National Clinical Guidelines In Paediatric Dentistry primarily, the identification of any related aetiology of dental erosion prior to any dental management should be emphasised in order to reduce the harmful effects of the risk factors through relevant information regarding life style and behaviour, presence of vomiting, medication to identify relevant extrinsic or intrinsic aetiological factors in addition to looking for any possible individual predisposing factors such as salivary rate and buffering capacity (O'Sullivan and Milosevic, 2008).

2.9.2 Early diagnosis

Early recognition of dental erosion may decrease the progress of the loss of dental tissue through careful examination of the most susceptible surfaces e.g. labial or palatal surfaces of upper of all upper teeth or occlusal surfaces of the lower first molar, cupping of cuspal tips or grooving of incisal edges and looking for symptoms of sensitivity or dental dysfunction and medical history (Gandara and Truelove, 1999).

2.9.3 Medical history

History of vomiting either spontaneous or self-induced, may be associated with a variety of medical problems e.g. children with neurological diseases such as cerebral palsy may have gastro-oesophageal reflex that may lead to dental erosion (O'Sullivan et al., 1998, Shaw and Smith, 1999).

2.9.4 Dietary counselling

Dietary questionnaire regarding the frequency and manner of intake of acidic food and drinks such as acidic beverages which are sipped over the longer period or habitually enjoying holding acidic drinks can cause a significant damage to the tooth surfaces (Gandara and Truelove, 1999, Shaw and Smith, 1999).

2.10 Fluoride as an oral health promoter

For many decades, fluoride has been considered as the effective element as an oral health promotion. Fluorides play a fundamental role in the prevention and controlling most of the dental diseases (Brambilla, 2001).

Fluoride ions have a strong affinity for exchanging with hydroxyl ions in hydroxyapatites because of its electronegative nature (F^-). The electrostatic attraction between calcium ions (Ca^{2+}) and (F^-) are considered greater than between (Ca^{2+}) and (OH^-), making the fluorapatite lattice more crystalline and stable (Posner et al., 1984). Exchange of fluoride ions in saliva are effective in shifting the balance from demineralisation, leading to remineralisation of the incipient carious lesions. This is attributed to the fluoride - enhanced precipitation of calcium phosphates, and the formation of fluorhydroxyapatite in the dental tissues. The presence of low fluoride levels in saliva after tooth brushing with fluoride containing dentifrices leading to ion exchange around the tooth surface. The rate of dissolution of mineral depends on pH, the actual concentrations of calcium and phosphate in the fluid in contact with the crystallites, and on the part of the layer covered by adsorbed fluoride (Arends and Christoffersen, 1990, ten Cate and Featherstone, 1991). The level of fluoride necessary for strong inhibition of enamel demineralisation *in vitro*

was estimated to correspond to a fluoride concentration in the liquid phase of 1 ppm or 50 mμmol/L fluoride ions (Arends and Christoffersen, 1990).

2.10.1 Systemic fluoride

Various varieties of systemic and topical fluorides have been presented into the public in order to prevent or treat dental diseases.

2.10.1.1 Water fluoridation

Water fluoridation is a controlled adjustment of fluoride supply to the communal drinking water to achieve a maximum caries reduction and insignificant level of dental fluorosis (Pizzo et al., 2007, Cho et al., 2014). It is considered an important safety and most cost – effectiveness public health measure in populations where oral hygiene conditions are poor, lifestyle results in a high caries incidence, and access to a well-functioning oral health care system is limited (Pizzo et al., 2007).

Water fluoridation at 1 ppm F level has been consistently demonstrated to be effective in reducing caries prevalence (Olofsson and Bratthall, 2000). It was suggested to reduce the concentration of fluoride in the domestic water supply to 0.6-0.8 ppm, with a range of 0.7 ppm to avoid the risk of fluorosis in children in case of toothpaste ingestion during the period of enamel formation and maturation stage (Whelton, 2007). When caries prevalence is high and the caries development is monitored over a long- term period a reduction of 40–49% in primary teeth and 50 – 59% in permanent teeth (Spencer, 1998, Limeback, 1999, Brambilla, 2001).

The advantages of water fluoridation of communal water was that it had a major effect on retaining more natural teeth among 35-44 year-olds compared to non-fluoridated area groups in 2000/’02. Furthermore, Children living in non-fluoridated

regions were 1.5 times more likely to have smooth surface wear compared with children in fluoridated regions and use of fluoridated toothpaste twice a day provide added protection from dental erosion (Bardsley et al., 2004). It was found that diffuse opacities were more prevalent among those with full water fluoridation than those without fluoridation (Whelton, 2007).

2.10.1.2 Alternative systemic fluoride delivery

Salt, milk and sugar, school water fluoridation and fluoride supplementation in the form of lozenges and tablets are suitable alternatives to domestic drinking water fluoridation and mainly containing sodium fluoride. The main advantage of most of these alternatives as a vehicle for fluoride delivery are that they do not require a community supply such as water and give individuals the freedom of choice (Brambilla, 2001) in terms of caries reduction, similar to those of fluoridated water (Mariño, 1995, Stephen et al., 1984).

Sucking fluoride tablets or lozenges offer direct fluoride action on the external surfaces of the teeth and give better results in caries reduction compared to swallowing tablets (Primosch et al., 1986, Hellwig and Lennon, 2004). Sucking acidic lozenges can cause erosion of the enamel (Lussi et al., 1998).

In areas with fluoridated drinking water the application of fluoride tablets is not advisable for toxicological reasons. The use of fluoride dentifrices by children living in these areas should be limited to those who are able to spit out adequately after tooth brushing (Hellwig and Lennon, 2004). The increase in the prevalence of fluorosis seen in recent years is due to an increase in the ingestion of fluoride from toothpaste by children during the period of amelogenesis. (Whelton, 2007).

2.10.2 Topical fluorides containing - products

Solutions, gels, varnishes, tooth-pastes and rinses of sodium fluoride, stannous fluoride, amine fluorides, acidulated phosphate fluoride and monofluorophosphate were introduced for topical fluoridation (Brambilla, 2001). Fluoride slow-release devices (Toumba and Curzon, 1993, Toumba and Curzon, 2005), or in the form of fluoride-releasing restorative materials (Wiegand et al., 2007, Forss, 1993), may release the fluoride material slowly into saliva and plaque to levels at which dental caries can be inhibited. Development of a chewing gum containing fluoride was tested for remineralisation of carious lesions (Hattab et al., 1988).

2.10.2.1 Fluoride rinses and solutions

Since1940s the most common fluoride solution was initially 2% sodium fluoride which was applied for 3 – 4 min after oral prophylaxis. Subsequently a number of other compounds were used such as stannous fluoride, acidulated phosphate fluoride and amine fluorides (Van Rijkom et al., 1998).

In recent years, in an attempt to find simple, time-saving and effective methods for fluoride application mouth rinses were developed in the 1950s, sodium fluoride would seem to be the common agent (Olofsson and Bratthall, 2000). The *in vitro* application of high concentration of 2,000 ppm sodium fluoride solutions on eroded dentine samples immediately before tooth brushing were found to have a better protection against abrasion of eroded dentine compared to 250 ppm sodium fluoride solution (Attin et al., 1998).

Tin-containing fluoride solutions were effective as anti-erosive agents in enamel at higher concentrations (1,900 mg/kg Sn as $SnCl_2$ (stannous chloride) compared to NaF solution (positive control, 1,000 mg/kg F In *an in situ* experiment (Schlueter et

al., 2009d). The efficacy of solutions with high concentrations of tin and fluoride were very effective in reducing erosive tissue loss in an *in vitro* study (Schlueter et al., 2009c).

2.10.2.2 Fluoride gels and varnishes

Fluoride varnishes provide a longer contact time between fluoride and the tooth surface, thereby improving fluoride incorporation into the surface layers of the enamel (Seppä, 2004). Experimental studies have shown that varnishes supply fluoride more efficiently than other topical agents. Fluoride varnish treatment effectively inhibits demineralisation, resulting in highly significant caries reductions, ranging from about 50 to 70% in fissures and an even higher percentage in the proximal surfaces (Seppä, 2004). Primary and permanent teeth revealed different F varnish and gel effectiveness when both were demineralised by cola (Murakami et al., 2009). The effect of sodium fluoride varnish (2.26%F) and APF gel on the erosive wear of primary and permanent enamel specimens were tested in six daily dem/remineralisation cycles eroded in a cola drink (pH 2.3) for 5 minutes. Both fluoride varnish and gel offered protections for permanent teeth only and were not significant in primary teeth (Murakami et al., 2009).

Daily application of topical gel (12,500 ppm F, partly as NaF, Olafluor and Dectafluor, pH 4.5) in combination with a fluoridated toothpaste (1,450 ppm F as NaF), gave increased reduction in demineralised enamel samples compared to fluoridated toothpaste alone (1,450 ppm F as NaF). After 4 weeks in the mouth, the reduction was 54% in the toothpaste + gel group and by 44% in the toothpaste-only group), but the difference between the groups was not statistically significant (Lagerweij and ten Cate, 2002).

The analysis of the protective effects of titanium TiF(4), zirconium ZrF(4), hafnium HfF(4) Tetrafluorides solutions (0.4 or 1%) and 1.25% AmF/NaF gel were tested on erosion of pellicle-free and pellicle-covered bovine enamel and dentine (20 specimens in each group) *in vitro*. Half the specimens in each group were immersed in human saliva for 2 h for pellicle formation. Specimens were then left untreated (controls) or were treated for 120 s with TiF(4), ZrF(4) or HfF(4) solutions (0.4 or 1%) or 1.25% AmF/NaF gel. All specimens were exposed to hydrochloric acid, pH 2.6, for 25 min. Cumulative calcium release into the acid was monitored in repeated 30-second intervals for 5 min, then at 2-min intervals up to a total erosion time of 25 min. The results showed that the specimens treated with 1% TiF(4) solution offered the best protective effect, especially in dentine (reduction of calcium loss about 50% at 25 min) compared to1% ZrF(4), 1% HfF(4) and 0.4% TiF(4). Long-term effects were limited to dentine, while 25% reduction of enamel erosion was noted with 1-min erosion. It was found that the fluoride gel had a protective effect only in dentine, whereas the efficacy of the tetrafluorides was influenced by the presence of the pellicle layer (Wiegand et al., 2008).

2.10.2.3 Fluoride compounds

There is an increasing amount of going knowledge about the erosion inhibiting potential of fluorides particularly of compounds with polyvalent metal cations (Ganss et al., 2012).

The effectiveness of a variety of fluoride compounds have been investigated extensively and gave different results against erosive or and as erosive / abrasive reductions. These include the effect of sodium, amine, stannous, tin containing fluorides, titanium fluoride and even zirconium and hafnium fluoride.(Wiegand et al.,

2009a, Wiegand et al., 2008). Sodium fluoride compounds have the longest practise and widely present in systemic and topical uses (Brambilla, 2001).

The prevention of enamel and dentine mineral loss was found to be effectively reduced by using intensive fluoridation with toothpaste (SnF_2/Olaflur; 0.14% F; Meridol®) in combination with a mouthrinse (SnF_2/Olaflur; 0.025% F; Meridol®) for 3 × 5 min daily and with a gel (NaF/Olaflur, 1.25% F; Elmex® gelee, GABA AG) compared to using fluoridated toothpaste (SnF_2/Olaflur; 0.14% F) during *in situ* study (Ganss et al., 2004a).

Pre-treatment with Naf and SnF_2 application in high concentration amounts did not appear to have protection against gastric erosion and tooth brushing abrasion (Austin et al., 2011).

The remineralisation performance of dentifrices under erosive conditions was significantly greater with the use of dentifrices containing sodium fluoride in the form of Sensodyne Pronamel (1450 ppm F as NaF/5% KNO_3) compared to compared to Blend-a-Med Classic(1450 ppm F as NaF); and Crest Cavity Protection (1100 ppm F as NaF); demonstrated significantly better relative erosion protection (% RER) than Crest Pro-Health (0.454% SnF2 [1100 ppm F]/sodium hexametaphosphate) (Barlow et al., 2009).

The SnF2/SHMP (0.454% stannous fluoride/ sodium hexametaphosphate) dentifrice (blend-a-med EXPERT GUMS PROTECTION) demonstrated inhibition of plaque regrowth both overnight and during the day considerably better than the NaF/KNO_3 dentifrice (Sensodyne ProNamel) (Bellamy et al., 2009).

Pre-treatment of enamel with carbamide peroxide10% CP (8 h) followed by fluoridation four times in 2000 ppm NaF solution does not improve erosive resistance (Burgmaier et al., 2002).

Different fluoride compounds were tested for their effectiveness as anti-erosive agents in human enamel samples that underwent a de- and re-mineralisation procedure for 10 days. The erosive challenge was achieved with 0.05 m*M* citric acid (pH 2.3) for 6 × 2 min daily followed by two minutes immersion in the test solution for 6 times. The test solutions were $SnCl_2$ (815 ppm Sn; pH 2.6), NaF (250 ppm F; pH 3.5), SnF_2 (250 ppm F, 809 ppm Sn; pH 3.5), amine fluoride (AmF, 250 ppm F; pH 3.5), AmF/NaF (250 ppm F; pH 4.3), and AmF/SnF_2 (250 ppm F, 390 ppm Sn; pH 4.2). Significant decrease in erosive mineral loss was revealed in the groups treated with $SnCl_2$ and NaF, whereas, AmF and AmF/NaF demonstrated lesser significant effect on erosion progression. The treatment of human enamel samples with solutions containing stannous fluoride (SnF_2) was most effective compared to those without a fluoridation solution (Ganss et al., 2008).

Evaluation of the effect of 1 and 4% titanium tetrafluoride (TiF4) gels, amine fluoride (AmF) 1 and 0.25% and a fluoride varnish (FP) on the prevention of dental erosion using bovine enamel samples submitted to alternate cycles of acid exposure in citric acid and remineralisation in artificial saliva. The cumulative erosion depth (µm) after 72 min was significantly lower for the group pre-treated with the fluoride varnish (FP-blanc) than other tested products ($p \leq 0.001$) (Vieira et al., 2005).

The addition of sodium fluoride to citric acid solutions leads to formation of surface CaF_2 and considerably reduces the changes in the apatite structure. But these

deposited CaF_2 globules seem to be insufficient to prevent the alteration of the apatite structure upon further exposure to acidic agents (Wang et al., 2008).

Erosive and abrasive wear were reduced by daily applications of high concentration fluoride gels either (amine/sodium fluoride gel, pH 4.8; 12,500 ppm), or sodium fluoride gel (pH 7.1; 12,500 ppm, irrespective of their fluoride compound, while the application of CPP-ACP-containing mousse was less effective (Wegehaupt and Attin, 2010).

Toothbrush abrasion for 30 seconds was not significantly lower using a single application of fluoride sodium/amine fluoride rinsing solution (250 ppm F) for 30 s *in situ* before or after softening the enamel in 0.1 M citric acid (pH 3.5) for 3 min compared to no rinsing (Lussi et al., 2004a).

The formation of surface coating after topical applications of titanium tetrafluoride TiF_4 on softened human enamel samples showed effective protection against hydrochloric acid exposures (Büyükyilmaz et al., 1997b).

The incorporations of metal cations into fluoride preparations in an attempt to protect against dental erosion progression under severe erosive challenge were studied in human enamel *in vitro*. Rinsing with iron solution for 1min with 10mL of a 10mM ferrous sulphate solution after an erosive exposure for 5min in 150mL of cola drink demonstrated a significant reduction on the %SMH in enamel (Sales-Peres et al., 2007). The addition of iron at 10 mmol/L into the demineralising solution significantly reduced the wear, nevertheless significantly improved the %SMHC of bovine enamel blocks submitted to erosion by Coke (Kato et al., 2007). The application of highly concentrated tin preparation (AmF/NaF/$SnCl_2$ that contained 2,800 mg/l Sn2+) was

able to reduce erosive enamel loss by 93.1%, even under severe erosive conditions (Schlueter et al., 2009a).

The effect of in situ brushing of volunteers teeth before insertion of the oral appliance with NaF 1,098 ppm F dentifrice for 1 minute 4 times a day and demineralisation of blocks of human enamel samples 4 times a day in a cola drink for 5 minutes showed no protection against erosion (Magalhaes et al., 2008b).

2.10.3 The importance of the fluoride toothpaste and tooth brushing

Fluoride toothpastes are the most universally self – applied agents and almost everyone uses a toothpaste in conjunction with tooth brushing (Marinho, 2009). Toothpastes are excellent vehicles for the delivery of fluoride because of widespread availability, and the main interest in toothpaste research is because it is the most widely accepted practical means of providing large groups of people with a regular supply of fluoride, in addition to its effectiveness and least risk of possible undesirable side-effects (Ekstrand, 1987, Baig and He, 2005). While the primary use of topical toothpaste substances with the tooth brush is to clean the accessible surfaces of the teeth, it may also deliver one or more of the additional benefits such as, primarily cosmetic, including cleaning, polishing and breath freshening, or secondly cosmetic – therapeutic through the efficient physical –mechanical removal of the dental plaque, and may provide therapeutic or pharmacological by means of transmission a treatment substance to the tooth surfaces or the surrounding tooth environment (Volpe, 1982, Attin and Hornecker, 2005).

The recommendation of twice daily tooth brushing is always advised by most dentists to maintain oral hygiene health (Attin and Hornecker, 2005). However, tooth

brushing performance after meals is always instructed, but with the current knowledge of potential harm of the altered softened tooth structure by brushing after recent intake of any erosive acidic foods and drinks giving such advise may be modified on an individual basis (Attin and Hornecker, 2005). It was suggested to leave 1 hour interval before brushing the teeth of an individual at risk of erosive wear after consuming any erosive foods or drinks (Jaeggi and Lussi, 1999). The efficacy of fluoride toothpastes is potentially influenced by several factors such as, fluoride concentration, frequency of use, amount used and rinsing behaviour (Davies et al., 2003).

Although, fluoride toothpastes are clinically proven to prevent and control dental caries (Hausen, 2004, Davies et al., 2003), its role in erosion and abrasion protection is still controversial. For instance, the calcium and fluoride-like material that may be deposited after topical fluoride application is assumed to be easily dissolved in most acidic solutions and also it has been shown that added saturated calcium – fluoride to 10 soft drinks and orange juice was unable to halt erosive lesions (Larsen, 2001).

Both *in vitro* and *in situ* studies on human as well as on bovine teeth have investigated the enamel surface loss subjected by acidic erosion or combined erosion and abrasion challenges. An *in situ* study (Hove et al., 2008) revealed that better protection against development of erosion-like lesions on human enamel by treatment with TiF_4 titanium tetrafluoride comparing to NaF treatment, whereas, (Magalhaes et al., 2008a) found that TiF_4 was unable to protect against dental erosion. In a study by Lagerweij et al., (2006), it was found that application of a highly concentrated acidic gel (12,500 ppm F) was able to protect against erosive and abrasive enamel wear, while using toothpaste alone with or without fluoride provided insignificant protection.

The erosion of human enamel by orange juice were exposed to the three regimens; an experimental toothpaste containing sodium hexametaphosphate, a benchmark sodium fluoride paste and a negative control, water, in a 15-day in situ single blind, crossover clinical model; and the same in an in vitro enamel erosion model. The depths of the experimental eroded areas were measured using a profilometer and there was significantly more erosive damage on the specimens exposed to the benchmark paste and water compared to the experimental paste in both the in situ and in vitro studies (Hooper et al., 2007).

The effect of topical fluoride applications in the form of gel (APF gel, 1.23%F) and varnish (NaF, 2.26%F) were studied on dental erosion in primary and permanent enamel specimens and showed the ability to inhibit erosion mainly in the permanent rather than primary teeth (Murakami et al., 2009).

A highly concentrated fluoride dentifrice tested *in situ* did not show a protective effect on enamel against erosion and combined erosion and abrasion when using a cola drink (60 seconds/ 4 times a day) and tooth brushing abrasion (30 seconds/4 times a day) (Rios et al., 2008b) while *in situ* studies demonstrated a high efficacy of fluoride toothpaste against enamel demineralisation under cariogenic challenge (Duggal et al., 2001, Zaura et al., 2005). Erosive challenge by a cola drink (3 times a day) for two weeks presented a significantly higher wear than by erosion + caries challenges (Honório et al., 2008).Fluoride solution containing titanium fluoride (TiF_4) was found to provide protection for enamel against acidic dental erosion (Hove et al., 2007) better than SnF2 and NaF. The topical treatment of human enamel with prevident (5000 ppm F) significantly increased enamel resistance to erosion by orange juice (Ren et al., 2009).

2.11 Use of bovine enamel for evaluation of dental erosion

Bovine enamel specimens have been used widely in recent dental research as a substitute for human enamel (Lagerweij et al., 2006, Rios et al., 2008b, Ruse et al., 1990). Bovine enamel has a number of advantages required for certain simple and straightforward methods, such as surface microhardness (Vieira et al., 2005), and represents a reproducible model for erosion experiments. Human enamel is becoming increasingly difficult to obtain and is of a highly variable composition when compared to bovine enamel (Mellberg et al., 1992).

2.12 Summary

Enamel erosive wear is characterised by acid-induced surface softening that, if unchecked, will progress to irreversible loss of surface tissue and potentially exposing the underlying dentine. The growing interest today in tooth surface loss is due to combined acidic erosion and abrasive wear commonly associated with tooth wear and affects a wide range of people. Brushing may be attributed to improving oral health, and to the presence of more teeth in an older population, a change of modern cultural life choices has led to excessive exposure to various exogenous acid sources and the hydrochloric acid regurgitated from the stomach that have the potential to contribute to the chemical erosion of tooth surfaces. Multiple risk and preventive factors may contribute to the severity and / or treatment of erosive wear.

3 Study 1 (part I): *In vitro* assessment of the effect of fluoridated toothpastes on bovine enamel subjected to erosion and abrasion

3.1 In vitro models for evaluation of dental erosion

The experimental conditions of *in vitro* methods can be carefully controlled, enabling straightforward modifications of model sensitivity and dynamic range to meet the standardised laboratory requirements (White, 1995). Although, *in vitro* studies do not reflect the physiological natural process in the human oral cavity (Hellwig and Lussi, 2001), the progress *in vivo* and *in situ* experimentation has led many investigators to speculate as to the relevance and importance of *in vitro* testing protocols in dental caries research (White, 1995).

As the process of erosive dental wear by acidic products and tooth brushing abrasion are observed frequently, efforts have been made to elucidate how erosive and abrasive lesions can be studied and prevented or reversed. Tooth brushing with fluoridated toothpaste is a worldwide accepted method of oral hygiene maintenance and responsible for the decline of dental caries (Twetman et al., 2003).

During recent years, an increasing number of various studies have suggested that dental erosion and tooth brushing abrasion may act synergistically to produce wear of tooth hard tissues (Jaeggi and Lussi, 1999, Eisenburger et al., 2003, Attin et al., 2004). The morphological changes of the dental surfaces with abrasion may be seen as diffuse or localised according to the principal impact and due to lower dentinal microhardness. Abrasion can be found on exposed coronal dentinal surfaces and root areas, whereas on occlusal surfaces, it is difficult to distinguish from erosion (Ganss et al., 2011b). A study by Larsen and Richards (2002) showed that fluoride treatment was unlikely to provide a preventive effect against erosion

because an acidic drink will rapidly dissolve accessible calcium fluoride removing the remaining traces of a previous topical fluoride treatment. The effect of time was investigated on enamel demineralisation during acidic erosion by using 0.3% citric acid, pH 3.3 at different exposure times and the depth of erosion lesions increased linearly with the exposure time (Eisenburger et al., 2001).

3.2 Aim and objectives

3.2.1 Aim

To assess the effect of various commercially available fluoridated toothpastes with different fluoride delivery systems and specific properties to target particular oral diseases as claimed by the manufacturers in the protection of bovine enamel subjected to *in vitro* cyclic acidic erosion and tooth brushing abrasion challenge.

3.2.2 Objectives

1. To evaluate the possible differences in tooth surface loss (µm) of bovine enamel after topical application of different delivery systems of fluoridated toothpastes using *in vitro* acidic cycling and tooth brushing abrasion.
2. To compare the effects of different specific properties of fluoridated toothpastes with similar fluoride concentrations in enamel surface loss subjected to acidic erosion and tooth brushing abrasion. To investigate the effectof the following fluoride products: Meridol® 1400 ppm F with AmF/SnF_2 as an anti-gingivititis agent and for gingival regeneration; Elmex® *anti-caries*, 1400 ppm AmF for caries protection and Elmex® sensitive plus, 1400 ppm AmF versus 0 ppm F toothpaste (Aronal®).

3. To compare Meridol® 1400 ppm F with AmF/SnF_2 for anti-gingivitis and for gingival regeneration; Elmex® *anti-caries*, 1400 ppm AmF for caries protection and Elmex® sensitive plus, 1400 ppm AmF versus Sensodyne pronamel® toothpaste 1450 ppm NaF + 5% potassium nitrate in enamel surface loss subjected to acidic erosion and tooth brushing abrasion.
4. To compare the results of this study with a similar study using human enamel (part II).
5. To establish experimental methodology to assess the effectiveness of fluoridated toothpastes for a longitudinal *in situ* model.

3.2.3 The null hypothesis to be tested for study 1

1. There are no differences between bovine enamel and human enamel on treatment and prevention of enamel surface loss subjected to 0.3% citric acid erosive / tooth brushing abrasive challenges under similar controlled *in vitro* conditions.
2. There are no differences between Amine fluoride containing toothpaste (Elmex® Sensitive plus; 1,400 ppm AmF, Elmex® *anti-caries* containing 1,400 ppm AmF, Meridol® toothpaste having combinations of 1,400 ppm AmF/SnF2 or NaF containing toothpaste (Sensodyne Pronamel® containing 1,450 ppm NaF +5% potassium nitrate in reducing enamel surface loss created by 0.3% citric acidic erosion and tooth brushing abrasion in comparison to 0 ppm F toothpaste.
3. There are no differences in the presence of the toothpastes containing similar concentrations of fluoride ppm F(Elmex® Sensitive plus 1,400 ppm AmF; Elmex® *anti-caries* 1,400 AmF; Meridol® toothpaste 1,400 ppm AmF/SnF2 in reducing the enamel loss created by acidic erosion and tooth brushing abrasion in comparison with Sensodyne pronamel® 1450 ppm NaF +5% potassium nitrate.

4. The effect of Meridol® toothpaste1,400 ppm AmF/SnF2 is not different from the other tested toothpastes containing similar concentrations of fluoride (Elmex® Sensitive plus; 1,400 ppm AmF and Elmex® *anti caries*;1,400 AmF in reducing enamel surface loss from acidic erosion and tooth brushing abrasion.

3.3 Materials and methods

3.3.1 Ethical approval

The source of enamel slabs was from bovine incisors which were collected from a local abattoir in agreement with government regulations.

3.3.2 Dental hard tissue substrates source and handling

Ten permanent lower incisors were obtained from young cattle at a local abattoir; the teeth were transported to the laboratory and stored in 0.1% thymol solution (Sigma Aldrich). In the laboratory, each tooth was held by a tweezer and residual calculus, bone, and soft tissues were removed with a disposable scalpel (Figure 3.1). The crowns were separated from the roots using a precision diamond wire saw, water cooled, cutting machine (Well® Walter EBNER, CH-2400 Le Loche) (Figure 3.2).

All crowns were cleaned with fluoride-free pumice and distilled water using a toothbrush, washed and stored in 0.1% thymol solution in a tightly sealed container at 4°C until required. Additionally, they were carefully checked for cracks, caries, or other malformations by visual inspection and transillumination and reflected light low power microscopy (Leitz, Wetzlar®, Germany).

For the experiment, the crowns of 10 teeth were cut mesio-distally, (Ruse et al., 1990).

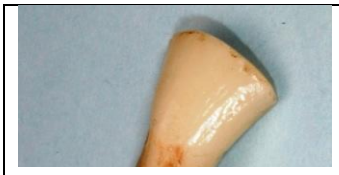


Figure 3.1 Freshly extracted lower bovine tooth

Figure 3.2 Well® Walter cutting machine with diamond saw wire

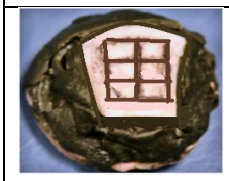


Figure 3.3 Mapping of the buccal surface of the tooth to enable sectioning into 6 specimens

3.3.3 Experimental design

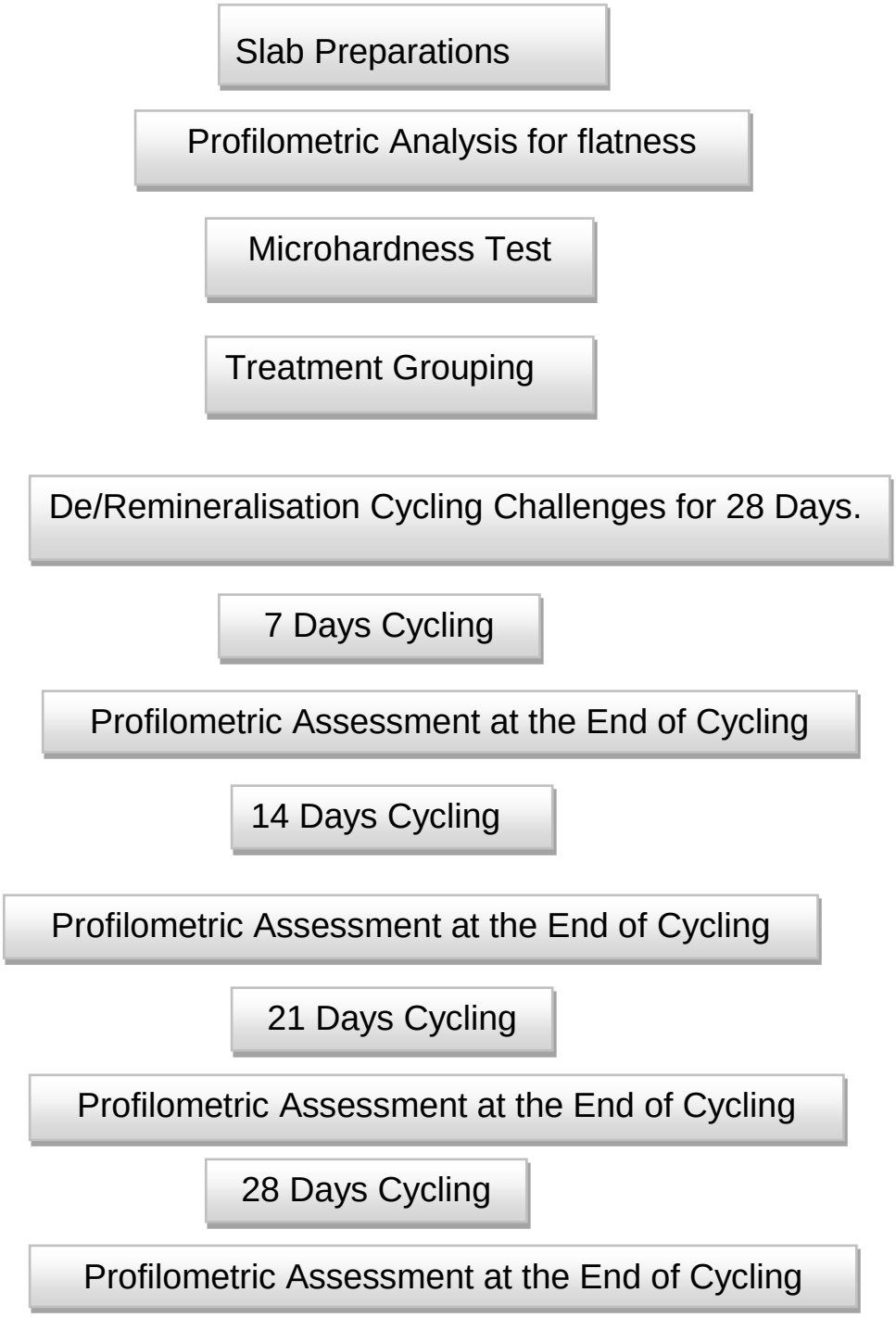
3.3.3.1 Mapping of the teeth

Each tooth surface was mapped with a pencil before cutting into 6 equal sections from the buccal surfaces of the 10 incisor bovine teeth. Five specimens from each tooth were used in the experimental groups and one was kept as a spare in case of loss or over grinding of one of the enamel slabs during slab preparations (Figure 3.3). The series of the sectioned enamel slabs from each tooth were arranged in resin blocks to constitute five experimental groups. Each experimental group comprised of 10 enamel specimens. Each enamel slab was identified and given three figures, the letter B which was referred to bovine enamel and two numbers (the number of the tooth and the slab number) e.g. (B11), so each enamel slab had a relevant identification figure for testing.

3.3.3.2 Storage of the enamel slabs

Once the slabs had been sectioned, they were kept moist in micro-centrifuge tubes containing de-ionised distilled water mixed with traces of thymol (0.1%) and kept at room temperature to prevent drying of the slabs. The five groups of the enamel slabs were incubated pre-experimentally in night time saliva at 37°C (as pre-conditioning prior to experimental procedures) to avoid dryness of prepared slabs and to simulate in vivo conditions

3.3.3.3 Flow chart



3.3.3.4 Slab preparations

Fifty enamel slabs (2x4 mm) were prepared from the extracted bovine incisors. They were mounted in circular resin discs of 3 mm thickness and 7.5 mm in diameter and ground flat with water-cooled wheel (Sic 600 and 1200 grades) and using a rectangular steel block which contained five circular holes of 3 mm depth to remove the outermost enamel remnants of the pellicle and to achieve a flat surface. Care was taken not to fully abrade the enamel. The mounting resin discs holding the enamel slabs had the same thickness and diameter of as the hole in the rotating steel block. This was followed by smoothening with very fine grit abrasive papers 2000 and 4000 grades (Wet or Dry paper, 3M) respectively to smooth the enamel surfaces. The slabs were then cleaned with methanol to remove any remnants of abrasive paper. The surfaces were then polished with 5 µm and 1 µm alumina paste. Thereafter, these slabs were cleaned with de-ionised distilled water and methanol to remove any remnants of abrasive paper or polishing material. Then each slab was covered with nail varnish (red colour, Max Factor®, England, UK) except for a small window that was left exposed. A special acrylic tray with 10 holes that fitted the resin blocks was used to hold the blocks. Then, every slab was secured in the hole within the acrylic plate with red adhesive wax.

3.3.3.5 Baseline laboratory measurements of the experimental slabs

To achieve baseline standardisation the experimental enamel slabs were assessed by profilometry scanning and Knoop microhardness testing.

3.3.3.5.1 Profilometry scanning

The baseline surface profile of the enamel samples were achieved by placing the dental tissue slabs on the key stage of the Proscan, Scantron profilometer surface with an adjustable measuring range having an average distance of 5 mm from the surface. The measuring rate/frequency was set to 300 Hz to give a minimum intensity of 5% of reflected light for analysis. The resolution of the sensor was 10 nm and the spot size was 8 µm with a step size of 0.01 mm (x) and 0.1 mm (y) was used during scanning (Figure 3.4). The profilometry dedicated software measured the mean level of the middle experimental area in relation to two reference areas using 3 level heights. The measuring range was set to 200 µm and after scanning, the flatness of the surface was checked by cross-sectional views and then the resultant values were expressed in micrometres (µm) (Hara and Zero, 2008). Slabs that were not flat were ground once again and rechecked by scanning. Slabs with dentine exposure were excluded.

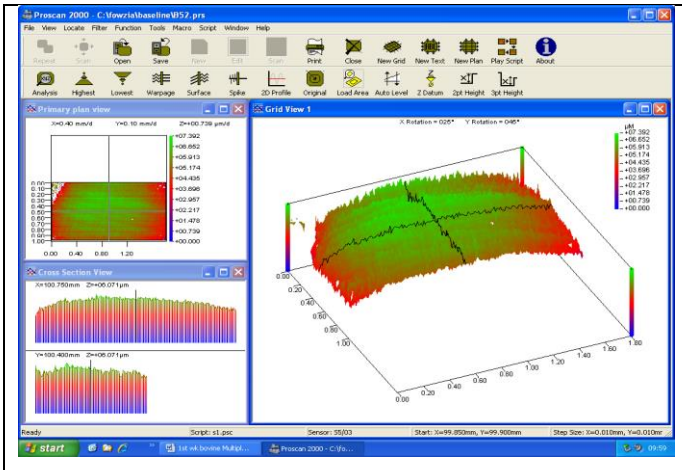


Figure 3.4 Checking the flatness of the enamel slab using profilometry (Proscan, Scantron 2000 UK)

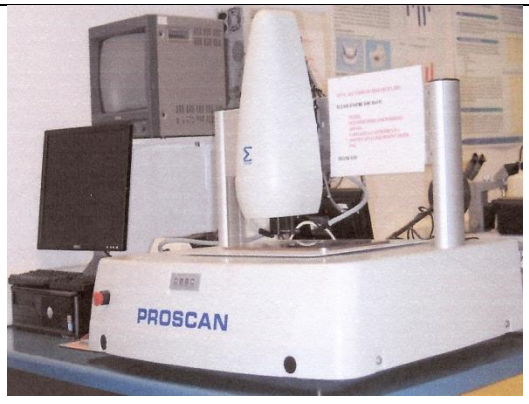


Figure 3.5 profilometry (Proscan, Scantron 2000 UK)

3.3.3.5.2 Knoop surface microhardness testing (KSMS) for baseline measurements

Surface microhardness was determined by creating three indentations on different surfaces of the slabs using a Knoop microhardness tester, with a 100 gm load for 15 seconds (Figures 3.6 and 3.7). The average of the 3 indentations was calculated.

Figure 3.6 Microhardness computer-aided Duramin indenter machine (Struers A/D, DK 26-19, Denmark)

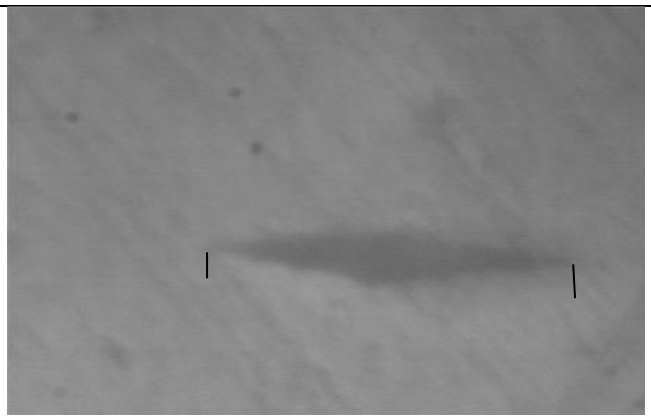


Figure 3.7 The indentation length in the enamel slab (µm)

Baseline microhardness was assessed using a Knoop microhardness indenter linked to a computer – aided Duramin Machine (Struers A/S, DK 26-10, Denmark) (Fig. 3.6). The length of the indenter penetration was measured by means of an image analysis system (Fig. 3.6). The length of the enamel indents were approximately between 61.5-66.9 µm which was considered as an acceptable hardness for bovine enamel (Appendix 1 table 8.1). After baseline measurements, the bovine enamel slabs were arranged into five groups with 10 specimens each. To maintain the reference surfaces for determination of lesion depth via profilometry, two layers of nail varnish (Max Factor®, England, UK) were applied on both sides to serve as reference areas and leaving a small experimental window exposed.

3.3.3.6 Erosive agent preparation

Citric acid was selected as the demineralising solution because of its high erosive potential that is attributed to its acidic nature and its ability to chelate calcium (Meurman and Frank, 1991). Most commercial beverages would usually have acidic concentrations of about 0.3% citric acid in their ready to drink juices (West et al., 2000, West et al., 2001). Therefore this percentage was chosen for the present study. The erosive challenge was for 2 minutes five times daily in citric acid 0.3% (pH 3.6). This was performed in the laboratory during a previous PhD study of Ahmed Abdullah who found that there were no differences between citric acid (0.3% pH 3.6), pure orange juice, or Sprite in creating erosive lesions and suggested the use of citric acid in subsequent studies. One of the main advantages of using citric acid is that it can be prepared freshly in the lab and does not contain any other chemical additives.

The demineralisation solution consisted of 0.3% citric acid adjusted to pH 3.6 by adding KOH (potassium hydroxide) solution. It was prepared using standard laboratory procedures using 15 grams of citric acid powder dissolved in 5 litres of de-ionised water in a sealed 5 litres volume plastic container and stirred using a stirrer machine (Heidolph, MR 3002, Germany) at room temperature. The samples were submersed sequentially into 50ml of 0.3% citric acid for 2 minutes five times per day at hourly intervals.

Citric acid required only titration of 3.1 mol with NaOH to bring the pH value to 7.0 at room temperature.

3.3.3.7 Artificial saliva preparations

Two types of artificial saliva, one for day time experiments (Table 3.1) and another for night time acting as storage for the enamel slabs after the experimental cycling per day during the whole cycling procedures period (Table 3.2) were prepared based on electrolyte composition of natural resting saliva for remineralisation studies. These two types of artificial saliva preparations were advised specifically to our laboratory study procedures for the PhD study by Ahmed Abdullah in 2009 by Dr P. Shellis (University of Bristol).

3.3.3.7.1 Preparation of day time artificial saliva

Table 3.1 Day time artificial saliva composition

Contents	Conc. g/l
Calcium carbonate	0.07
Magnesium carbonate (hydrated basic)	0.019
Potassium di-hydrogen phosphate	0.544
HEPES buffer (acid form)	4.77
Potassium chloride	2.24

The above materials were mixed in 900 ml of distilled water and 1.8 ml 1mol/l HCl, then, the mixture was stirred until all components had dissolved during which the pH was adjusted to 6.8 by adding KOH solution. The artificial saliva was stored in the refrigerator and used within a few days.

3.3.3.7.2 Preparation of night time artificial saliva

Night time synthetic saliva was used for storage of enamel slabs after the last experimental brushing.

Table 3.2 Night time artificial saliva composition

Contents	Conc. g/l
Calcium carbonate (CaCO3)	0.05
Magnesium carbonate (hydrated basic) MgCO3	0.019
Potassium di-hydrogen phosphate (KH2PO4)	0.068
HEPES buffer (acid form)	4.77
Potassium chloride (KCL)	2.24

The above components were mixed with 900 ml of distilled water with the addition of 1.4 ml 1 mol/l and stirred until all the components had dissolved. The pH was adjusted to 6.8 by adding KOH solution. The saliva was stored in the refrigerator and was used within a few days.

3.3.3.8 Test products

Five dentifrices (all from well-established European manufacturers) were included in the present study; four of them were provided by (GABA International AG, Grabetsmattweg, CH-4106 Therwil, Switzerland) with different specific properties (Table 4.3) and divided into 5 experimental treatment groups (Table 3.4); they were as follows:

- E1 Meridol® containing amine and stannous fluoride (AmF/SnF_2, 0.14% F).
- E2 Elmex® *anti-caries* containing amine fluoride (AmF, 0.14% F).
- E3 Sensodyne Pronamel® (GlaxoSmithKline, Brentford, TWB GSK, UK) against acid erosion contains sodium fluoride (NaF, 0.14. 5% F).
- E4 Elmex® Sensitive Plus amine fluoride (AmF, 0.14%).
- E5 Aronal® (0 ppm F) (as control group).

Table 3.3 summarises the detailed fluoride delivery system and specific properties for each experimental product.

3.3.3.9 Randomisation

Fifty bovine enamel slabs were sectioned from 10 permanent lower incisors of young cattle and randomly allocated into 5 groups. Each group comprised of 10 enamel slabs and in each group one slab from each tooth was present within the group.

3.3.3.10 Blindness

The test products were not indistinguishable to the study researcher because they could not be “blind” during the application of the test products throughout the whole period of the experimental procedures. On the other hand, the researcher was “blind” when measuring the enamel surface loss by profilometry.

3.3.3.11 Reproducibility:

Re-evaluation of 20% of the slabs using the surface profilometry test was carried out randomly by the study investigator to check the reproducibility at different timings and after switching off the software device to carry out the other 2^{nd} reading (appendix 20). The average of three readings was taken for each slab by the profilometry scanning (Proscan, Scantron, 2000, UK). The measurements were re-evaluated by another examiner.

Table 3.3 Test products delivery system and related specific properties

Fluoride (%)	Source	Trade name	Specific properties	Target group	Abrasive type	**Other key components**
0.14% F	AmF/SnF_2	Meridol®	Anti-gingivitis, inactivate residual plaque and inhibits new plaque formation and additionally protects against caries	People with irritated gums	Hydrated silica, Silica Dimethyl Silylate	Cocomidopropyl Betaine, PEG-3 Tallow Aminopropylamine, Hydrochloric acid.
0.14% F	AmF	Elmex® *anti-caries*	Caries protection and remineralisation of initial caries (mineralisation of dental enamel)	People with permanent teeth for daily caries protection	Hydrated silica	Hydroxymethylcellulose
0.14.5% F	NaF	Pronamel®	Against erosion and helps re-harden enamel.	People at risk of dental erosion	Low abrasivity	Neutral pH formula with 5% w/w Potassium nitrate.
0.14%F.	AmF	Elmex® sensitive plus	Protection against cervical caries and dentine sensitivity, gentle cleansing action	People with exposed and hypersensitive dentine	Hydrated silica, Silica Dimethyl Silylate	Hydroxymethylcellulose, polyethelene
0 ppm F	medicated	Aronal®	Strengthens the gum gently removes plaque and at the same time that massages the gums slightly	People who are sensitive to fluoride or very young children	Silica	Dicalcium phosphate Dihydrate. Allantoin and Aluminium lactate Propylene Glycol Glycerine

3.3.3.12 Treatment grouping

There were five treatment groups, each consisting of an acrylic block with 10 holes holding 10 enamel slabs from each tooth, (10 specimens in each group). All toothpaste test groups (Table 3.4) were subjected to brushing abrasion during toothpaste application and acid cycling challenge for 2 minutes on 5 occasions per day.

Table 3.4 Treatment groupings

Group	Treatment
E1 MERIDOL®	Slurry from amine AmF + Stannous Fluoride SnF2 (0.14% F) / day time artificial saliva formulation (pH 5.1) was applied for 2 mins and 15 strokes under 300 g load tooth brushing procedure.
E2 ELMEX® *anti-caries*	Slurry from amine fluoride AmF (0.14% F) / day time artificial saliva formulation (pH 5.2) was applied for 2 mins and 15 strokes under 300 g load tooth brushing procedure.
E3 PRONAMEL®	Slurry from sodium fluoride, NaF (0.1450 ppm F) / day time artificial saliva formulation (pH 6.7) was applied for 2 mins and 15 strokes under 300 g load tooth brushing procedure.
E4 ELMEX® SENSITIVE PLUS	Slurry from Olaflur (AmF) (0.14% F) / day time artificial saliva formulation (pH 5.8) was applied for 2 mins and 15 strokes under 300 g load tooth brushing procedure.
E5 ARONAL®(F-Free) as a control	Slurry from fluoride-free toothpaste / day time artificial saliva formulation (pH 6.6) was applied for 2 mins and 15 strokes under 300 g load tooth brushing procedure.

3.3.3.13 Preparation of toothpaste slurries

Toothpaste slurries were prepared for each experimental product in the ratio of 1:3 by measuring 5 g of each product mixed with 15 ml of day time artificial saliva in a 20ml plastic tube using a balance, and then, allowed to form a homogenous mixture with a Whirell mixer for 5 mins. The pH of the test toothpaste slurry was determined using a pH electrode (VWR international Orion, Orion research, UK) by adding NaOH (sodium hydroxide) solution until

the pH reached 3.6 at room temperature calibrated with 7.0 and 4.0 pH standards. These were prepared fresh on a daily basis. The pH of the toothpaste slurries showed distinctive variations (pH range from 5.1 to 6.7).

3.3.3.14 The tooth brushing technique

The tooth brushing machine (NEL-BS, Dentifrice Test Machine) consisted of four separate brushing basins each connected with a motor-driven brushing head. Each brushing head contained one soft toothbrush head Oral-B Orthodontic Braces Speciality Toothbrush (Appendix 3 in Fig. 8.1). The brushing head was connected via a joint to a metallic bar driven by an electric motor which moved the brushing head horizontally across the brushing basin one forward and backward, this brushing cycle was referred to as a 'stroke'. Each brushing basin well was labelled with the corresponding toothpaste and each experimental enamel group. Specimens were placed to ensure the enamel experimental windows faced up during which the toothpaste slurry application for two minutes and 15 strokes under 300 g loads were applied. As the brushing machine had only four separate basins, the 5^{th} group brushing head with its soft toothbrush head was replaced with that of the 1^{st} group after washing thoroughly with tap water.

3.3.3.15 De / remineralisations cycling procedures

In the pre-experimental day, all groups were stored in night time saliva before proceeding with the erosion and abrasion challenge. Erosive demineralisation was performed by immersion in 0.3% citric acid for 2 min (2x5) per day followed by incubation in day time artificial saliva at 37° C in between. Toothpaste slurries were applied two times daily for 2 min and 15 strokes under 300 g loads tooth brushing during toothpaste application (Table 3.5).

Rinsing with de-ionised distilled water was performed immediately in between the immersions in citric acid solution and after toothpastes applications. At the end of the cycling experimental period, the enamel slabs were collected and kept in a solution of 0.1% thymol mixed with distilled de-ionised water solution until analysis.

Table 3.5 Daily de/remineralisation regime for 28 days

Morning brushing (2min)	The erosive challenge of the experimental enamel slabs by dipping in 0.3 citric acid solution for 2 mins x 5 times per day					Evening brushing (2min)
Application of test toothpaste slurry (1:3) plus brushing 15 strokes 300 g load	Dipping in citric acid for (2min) followed by incubation in artificial saliva (day time) (1hr)	Dipping in citric acid for (2min) followed by incubation in artificial saliva (day time) (1hr)	Dipping in citric acid for (2min) followed by incubation in artificial saliva (day time) (1hr)	Dipping in citric acid for (2min) followed by incubation in artificial saliva (day time) (1hr)	Dipping in citric acid for (2min) followed by incubation in artificial saliva (day time) (1hr)	Application of test toothpaste slurry (1:3) plus brushing 15 strokes 300 g load Then storing slabs in storage saliva (night time)

3.4 Results of study (part I)

3.4.1 Profilometric analysis of bovine enamel surface loss (µm) (Proscan 2000, UK)

The depth of enamel surface loss (µm) was determined profilometrically from the reference areas on both sides and crossing the exposed experimental surface in the middle. The starting point of the scan was adjusted at the periphery of one side of the reference area crossing the middle experimental area to the other side of reference area using 3 level heights. The measurements were repeated three times and then taking the average of the three readings of every enamel slab to detect the enamel surface loss (µm) changes caused by 0.3% citric acid and tooth brushing abrasion. An example of some of the profilometric scans are displayed in (Appendix 4).

Using a cycling model of erosive challenge with 0.3% citric acid (pH 3.6) for 2 mins x 5 times per days and tooth brushing abrasion for 2 mins x 2 times per day showed considerable enamel wear depth (µm) in relation to both reference areas in the bovine enamel treated with Meridol® (AmF/SnF2, 1400 ppm F) Figures (appendices 8.2 and 8.3). Less Enamel erosive / abrasive wear depths (µm) were found in enamel slabs treated with Elmex® *anti-caries* (AmF, 1400 ppm F) as shown in Figures (appendices 8.4 and.8.5), Elmex® Sensitive Plus (AmF, 1400 ppm F) as shown in Figures (appendices 8.6 and 8.7) and Sensodyne® Pronamel (NaF, 1450 ppm F) as shown Figures (appendices 8.8 and 8.9). Figures 8.10 and 8.11 show some examples of enamel slabs treated with 0 ppm F toothpaste (Control) causing more enamel wear depth in comparison to enamel samples treated with Elmex® groups and Sensodyne® Pronamel toothpaste.

3.4.2 The distribution of the effect of testing five toothpastes on bovine enamel surface loss (µm)(part I)

3.4.2.1 The effect of five different toothpaste treatments on bovine enamel surface loss (µm) subjected to 0.3% citric acid erosion and tooth brushing abrasion during experimental periods at 7,14, 21 and 28 days.

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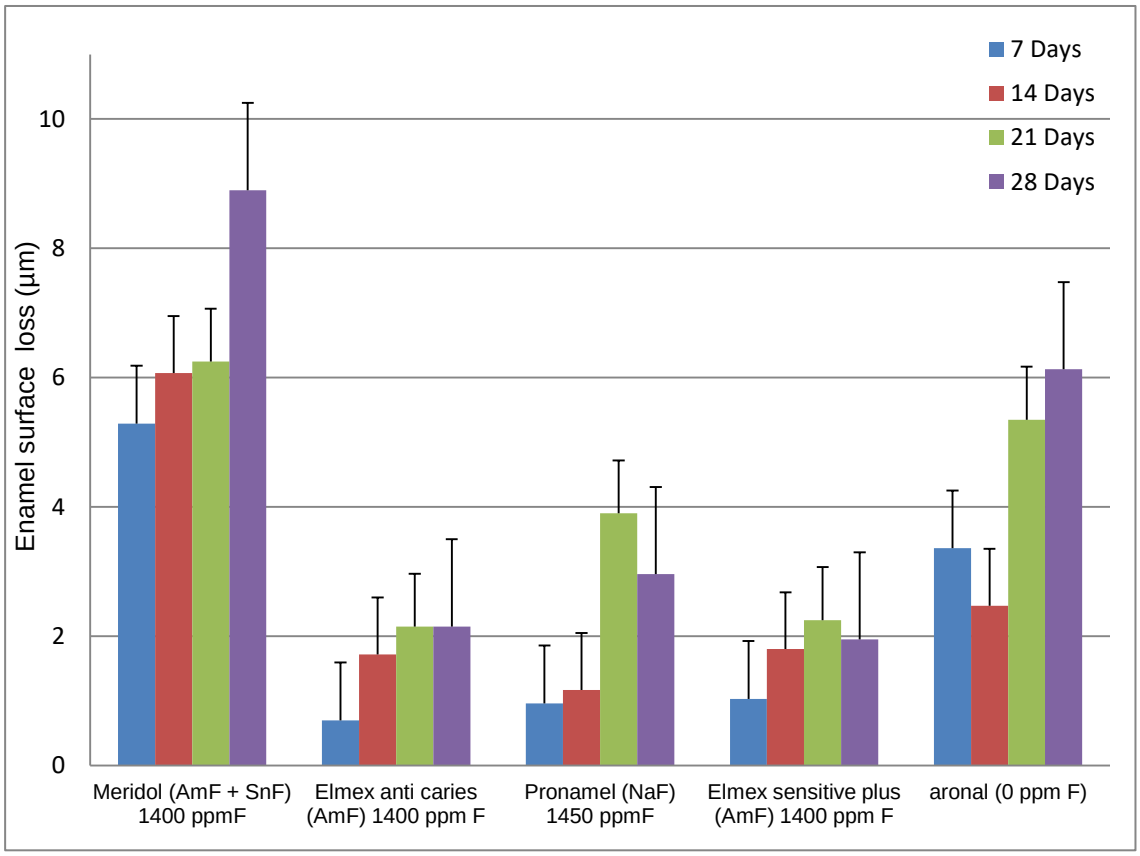


Figure 3.8 Comparisons between the effects of the five test toothpaste treatments on bovine enamel surface loss (µm) during the experimental periods of the study at days 7, 14, 21 and 28.

In Figure 3.8, all groups showed enamel surface loss (µm) which increased with repeated experimental cycling over the study periods (7, 14, 21 and 28 days) compared to baseline measurements. The greatest ESL (µm) was observed in the Meridol® (AmF/SnF) group, then the Aronal® (F-Free Control) group. Both Elmex® groups containing AmF, 1400 ppm F (Elmex® *anti-caries* and Elmex® Sensitive Plus) in addition to Sensodyne® Pronamel toothpaste (NaF, 1450

ppm F) showed less reduction in enamel surface wear after 7, 14, 21 and 28 days of cycling procedures.

4.2.2 Statistical analysis of the bovine enamel surface loss (µm)

There were five experimental groups which underwent four phases with each phase for one week; hence the objectives of analysis were as follows:

The data values were recorded according to the numerical code and distributed accordingly to the five groups. Evaluation was performed with the Statistical Package of Social Science (SPSS) at The University of Leeds computer system. The normal distribution of the data was tested with the Kolmogorov-Smirnov test. The clinically significant results within the groups were tested with t-tests for paired samples. For multiple comparisons between groups One-way analysis of variance (ANOVA) with a level of significance at $p \leq 0.05$ was used.

4.2.2.1 The distribution of data

Normality was checked (by Kolmogrov-Smirov test). The data were normally distributed when 2-tailed $p>0.05$ (Appendix 5 in Tables 8.1-8.4). The data distributions were found to be normal for all groups during the four phases of the experimental procedures; therefore One Way ANOVA tests were used for the statistical analyses.

4.2.2.2 Significance tests for ANOVA

Significance tests for ANOVA are presented in (Appendix 6 in Tables 8.7-8.10). In order to reject the null hypothesis the p value should be $p<0.05$. If the null hypothesis is rejected, the comparisons between any two groups at a time are

possible by using post hoc (LSD) tests. For multiple comparisons, as for the data in this study for which there are 10 comparisons, initially the significance level was set at 0.05 using (post hoc, LSD at 0.05), then for Bonferroni correction the significance level was calculated by α=0.005, and hence a new p value at 0.005.

4.2.2.3 Changes of bovine enamel surface loss (µm) shown in boxplots for all the experimental groups

Data analysis: Boxplots for bovine enamel surface loss (BESL) (µm). Boxplots (3.9-3.12) show the median, upper and lower quartile changes in ESL (µm) for each group during erosive-abrasion procedures for 7, 14, 21 and 28 days. The boxes demonstrate the quarter (0.25) and fourth quarter (0.75) percentile with the inner line in the box representing the median. Minimum and maximum values are represented by the horizontal lines outside the box.

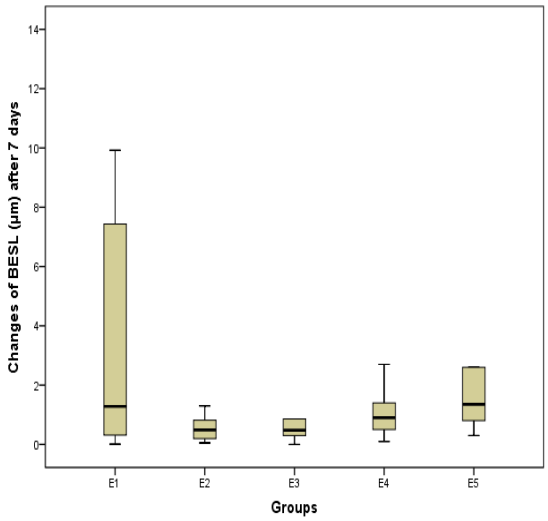


Figure 3.9 Changes in bovine enamel (µm) surface loss after 7 days

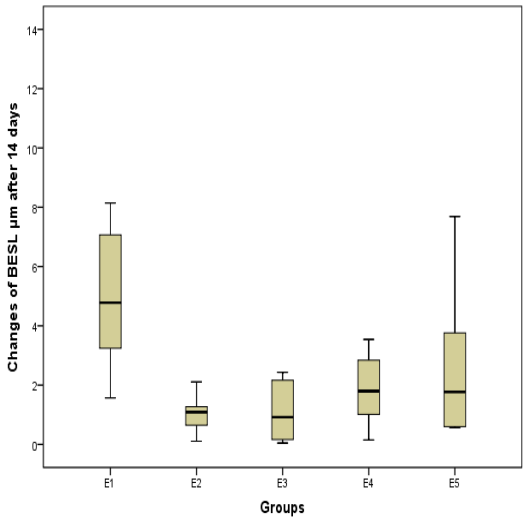


Figure 3.10 Changes in bovine ESL (µm) after 14 days

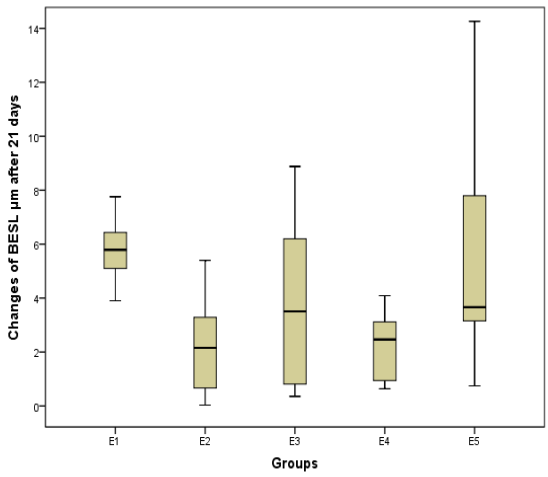


Figure 3.11 Changes in bovine ESL (µm) after 21 days

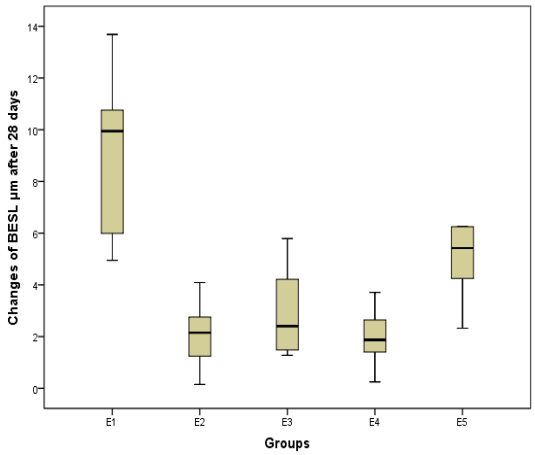


Figure 3.12 Changes in bovine ESL (µm) after 28 days

Changes of bovine enamel surface loss (BESL) (µm) from baseline achieved by the five different toothpaste treatments for twice daily erosive challenge using 0.3% citric acid for two minutes on five groups of experimental products.

In Figure 3.9, the bovine enamel surface loss (µm) changes are almost the same for all groups except for a slight variation for Meridol® (E1) and Aronal® (E5). Figure 3.10 shows that at 14 days cycling all groups except Meridol® (E1) showed similar median changes. Figure 3.11 showed that after 21 days both

Elmex® groups (E2 and E4) showed similar patterns of ESL (µm). Figure 3.12 showed that after 28 days both Elmex® groups (E2 and E4) had the same median changes and Pronamel® (E3) had a smaller difference compared to both Elmex® groups.

4.2.2.4 Distribution of the changes in the enamel surface loss (µm)

Table 3.6 displays the mean ± std. deviation (µm) median, minimum and maximum values for all the study groups at the end of 7, 14, 21, 28 days. All experimental groups showed different values of enamel surface loss compared to baseline.

Table 3.6 Distribution of the changes of bovine enamel surface loss (µm) achieved by 0.3% citric acid erosion and tooth brushing abrasion during the experimental cycling procedures

Cyclic Period	Toothpastes test groups	Mean (µm)	Std. Dev.	Median	Range	Interquartile range	Std. error	Min.	Max.
7 Days	Meridol® (AmF/SnF_2; 1400 ppm F) E1	*3.7*	*4.0*	*1.30*	*9.9*	*7.7*	*1.3*	*.01*	*9.92*
	Elmex® *anti-caries* (AmF; 1400 ppm F) E2	*0.70*	*.77*	*0.49*	*2.6*	*.80*	*.25*	*0.05*	*2.61*
	Sensodyne® Pronamel (NaF; 1450 ppm F) E3	*0.96*	*1.3*	*0.48*	*4.3*	*.91*	*.41*	*0.00*	*4.28*
	Elmex® sensitive plus (AmF; 1400 ppm F) E4	*1.03*	*.83*	*0.93*	*2.6*	*1.13*	*.26*	*0.08*	*2.70*
	Aronal® (0 ppm F) E5	*3.4*	*5.2*	*1.35*	*16.7*	*2.8*	*1.6*	*0.33*	*17.1*
14 Days	Meridol® (AmF/SnF_2; 1400 ppm F) E1	*5.0*	*2.4*	*4.78*	*6.6*	*4.3*	*.80*	*1.6*	*8.1*
	Elmex® *anti-caries* (AmF; 1400 ppm F) E2	*1.7*	*1.7*	*1.2*	*5.0*	*2.1*	*.53*	*0.11*	*5.0*
	Sensodyne® Pronamel (NaF; 1450 ppm F) E3	*1.2*	*1.00*	*0.92*	*2.4*	*2.1*	*.32*	*0.07*	*2.4*
	Elmex® sensitive plus (AmF; 1400 ppm F) E4	*1.8*	*1.20*	*1.80*	*3.4*	*2.1*	*.38*	*0.15*	*3.5*
	Aronal® (0 ppm F) E5	*2.5*	*2.3*	*1.77*	*7.1*	*3.2*	*.72*	*.60*	*7.7*
21 Days	Meridol® (AmF/SnF_2; 1400 ppm F) E1	*6.3 2.2*		*5.80*	*7.4*	*2.5*	*.74*	*3.90*	*11.3*
	Elmex® *anti-caries* (AmF; 1400 ppm F) E2	*2.1*	*1.7*	*2.2*	*5.4*	*2.9*	*.55*	*0.03*	*5.40*
	Sensodyne® Pronamel (NaF; 1450 ppm F) E3	*2.63*	*3.27*	*3.51*	*5.84*	*3.41*	*.65*	*0.36*	*6.20*
	Elmex® sensitive plus (AmF; 1400 ppm F) E4	*2.10*	*1.19*	*2.46*	*3.45*	*1.95*	*.35*	*0.64*	*4.09*
	Aronal® (0 ppm F) E5	*4.36*	*2.75*	*3.66*	*13.51*	*5.36*	*1.29*	*0.75*	*14.3*
28 Days	Meridol® (AmF/SnF_2; 1400 ppm F) E1	*8.71*	*3.10*	*9.59*	*8.97*	*8.97*	*1.03*	*4.95*	*13.7*
	Elmex® *anti-caries* (AmF; 1400 ppm F) E2	*2.10*	*1.13*	*2.15*	*3.94*	*1.58*	*.36*	*0.15*	*4.09*
	Sensodyne® Pronamel (NaF; 1450 ppm F) E3	*2.86*	*1.56*	*2.40*	*4.40*	*2.90*	*.49*	*1.28*	*5.79*
	Elmex® sensitive plus (AmF; 1400 ppm F) E4	*1.95*	*1.01*	*1.87*	*3.46*	*1.39*	*.32*	*0.25*	*3.71*
	Aronal® (0 ppm F) E5	*6.03*	*3.08*	*5.42*	*10.54*	*2.92*	*.97*	*2.33*	*13.90*

4.2.2.5 ANOVA results among the experimental groups

Results following ANOVA analyses are summarised in Table 3.7. These results showed that there were no significant differences at day 7 $p \geq 0.05$, whereas at days 14, 21 and 28 duration highly significant differences between the treatment groups were observed $p \leq 0.05$. Therefore the null hypotheses at days 14, 21 and 28 were rejected.

Table 3.7 ANOVA significance tests between and within the groups during the experimental procedures at 7, 14, 21 and 28 days.

Duration		Sum of Squares	df	Mean Square	P value
7 Days	Between Groups	72.65	4	18.16	.08
	Within Groups	364.56	44	8.29	
14 Days	Between Groups	62.78	4	15.70	.001
	Within Groups	127.44	44	2.90	
21 Days	Between Groups	146.60	4	36.65	.001
	Within Groups	264.80	44	6.02	
28 Days	Between Groups	328.95	4	82.24	.000
	Within Groups	204.24	44	4.64	

Pairwise comparisons between the groups using Least Significant Difference (LSD) showed significantly higher values for surface loss for Meridol and lower values for other groups ($p<0.05$). Further interpretation of the results using Bonferroni tests at a level of $p \leq 0.005$ was determined.

Table 3.8 shows no significant enamel surface loss (µm) difference among the treatment groups $p > 0.05$.

Table 3.8 The ANOVA significance test of the experimental procedure after 7 days

ANOVA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	82.330	4	20.582	2.297	.08
Within Groups	394.236	44	8.960		
Total	476.566	48			

$p > 0.05$ after 7 days of the experimental cycling procedures

In Table 3.9, as these were multiple comparisons, showed significant mean change differences (µm) with lower surface loss for Elmex® *anti-caries*, Elmex® Sensitive plus and Sensodyne Pronamel® compared to Meridol® p≤0.001 and Meridol® and Aronal® p ≤ 0.01. The mean difference (µm) for Aronal® with the other treatment groups was not significant.

Table 3.9 Comparisons between the five test toothpastes on bovine enamel surface loss (µm) achieved by erosive challenge in 0.3% citric acid and tooth brushing abrasion after 14 days cycling procedures.

Pairwise comparisons Between the groups		Mean Difference (µm)	Std. Error	P. value	95% Confidence Interval	
Meridol® E1	Elmex® *anti-caries* E2	3.25*	1.2	.001	1.97	6.73
	Elmex® Sensitive E4	3.27*	1.2	.001	1.89	6.65
	Aronal® F-Free E5	3.61*	1.2	.004	1.2	5.99
Elmex® *anti-caries* E2	Pronamel E3	0.55	1.2	.637	-1.8	2.86
	Elmex Sensitive E4	0.08	1.2	.945	-2.4	2.24
Pronamel® E3	Meridol E5	3.90*	1.2	.000	2.5	7.28
Elmex® Sensitive E4	Elmex® *anti-caries* E2	0.10	1.2	.945	-2.24	2.40
	Pronamel® E3	0.63	1.2	.600	-1.69	2.94
Aronal® F-Free Control E5	Meridol® E5	3.61*	1.2	.004	-5.99	-1.23
	Elmex® *anti-caries* E2	0.75	1.2	.519	-1.57	3.06
	Pronamel® E3	1.29	1.2	.267	-1.02	3.61
	Elmex® Sensitive E4	0.67	1.2	.565	-1.65	2.98

* The mean difference was significant at the 0.05 level.

In Table 3.10, both Elmex® *anti-caries* and Elmex® Sensitive plus continued to show significant mean difference changes (µm) with lower surface loss for Elmex® *anti-caries*, Elmex® Sensitive plus $p \leq 0.001$ and Sensodyne Pronamel® where the significance was high $p \leq 0.003$ compared to Meridol® $p \leq 0.001$. No significance was observed between Meridol® and Aronal®. The mean difference (µm) for Aronal® with the other treatment groups was significant at $p \leq 0.01$.

Table 3.10 Comparisons between the five test toothpastes on bovine enamel surface loss (µm) achieved by erosive challenge using 0.3% citric acid and tooth brushing abrasion after 21 days cycling procedures.

Pairwise comparisons Between the groups		Mean Difference (µm)	Std. Error	p value	95% Confidence Interval	
Meridol® E1	Elmex® *anti-caries*	4.11*	1.2	.002	1.59	6.63
	Elmex® Sensitive plus	4.01*	1.2	.002	1.49	6.52
	Aronal® F-Free	1.00	1.2	0.472	-1.61	3.42
Elmex® *anti-caries* E2	Pronamel®	1.75	1.2	.156	-4.20	0.70
	Elmex® Sensitive plus	0.10	1.2	.933	-2.55	2.35
Pronamel® E3	Meridol®	2.36	1.2	.066	-0.16	4.87
Elmex® Sensitive plus E4	Pronamel®	1.65	1.2	.182	-4.10	0.80
Aronal® F-Free E4	Elmex® *anti-caries*	3.20*	1.2	.012	0.76	5.65
	Pronamel®	1.45	1.2	.238	-1.0	3.90
	Elmex® Sensitive Plus	3.10*	1.2	.014	.65	5.55

* The mean difference was significant at the 0.05 level.

Table 3.11 shows Elmex® *anti-caries* (E1), Pronamel® (E2) and Elmex® Sensitive (E4) had less surface loss compared to Meridol® (SnF_2/AmF) which was statistically significant using Bonferroni test ($p \leq 0.001$). Also, both Elmex® groups and Pronamel® showed statistically significant differences compared to Aronal (0 ppm F) ($p \leq 0.005$). Meridol® compared to Aronal® showed no significance ($p > 0.05$).

Table 3.11 Comparisons between the five test toothpastes on bovine enamel surface loss (µm) achieved by erosive challenge in 0.3% citric acid and tooth brushing abrasion after 28 days cycling procedures

Pairwise Comparisons		Mean Difference (µm)	Std. Error	p value	95% Confidence Interval	
Meridol® E1	Elmex® *anti-caries* E2	6.80*	.98	.000	4.83	8.77
	Elmex® Sensitive plus E4	6.94*	.98	.000	4.97	8.91
	Aronal® F-Free E5	2.76*	.98	.007	.794	4.73
Elmex® *ant-caries* E2	Pronamel E3	0.86	.95	.369	-2.78	1.05
	Elmex® Sensitive plus E4	0.14	.95	.884	-1.78	2.06
Pronamel® E3	Meridol® E1	5.94*	.98	.000	3.97	7.91
Elmex® Sensitive plus E4	Elmex® *anti-caries* E2	0.14	.95	.884	-2.06	1.78
	Pronamel® E3	1.00	.95	.297	-2.92	.914
Aronal® F-Free E5	Elmex® *anti-caries* E2	4.04*	.95	.000	2.12	5.96
	Pronamel® E3	3.18*	.95	.002	1.26	5.09
	Elmex® Sensitive plus E4	4.18*	.95	.000	2.26	6.10

* The mean value was significant at the 0.05 level.

4 Study (part II): *In vitro* assessment of the effect of fluoridated toothpastes on human enamel subjected to erosion and abrasion

4.1 Aim:

To assess the effects of topical application of fluoridated toothpastes on human dental enamel subjected to both erosion and abrasion.

4.2 Objectives:

4.2.1 Primary objective:-

The primary objective was to evaluate the effects of topical application of fluoridated toothpastes in the form of different delivery systems for the prevention of enamel loss.

4.2.2 Secondary objectives:-

The secondary objective was to verify whether the effectiveness of fluoridated toothpastes is influenced by the erosive and abrasion challenges during 28 days (prolonged exposure of experimental challenges).

4.3 Null hypothesis

The null hypothesis was that there were no differences in the effectiveness of different test fluoride toothpastes on human enamel surface loss subjected to acidic erosion and tooth brushing abrasion challenge.

4.4 Materials and methods (part II)

4.4.1 Ethical considerations

Ethical consent no: 031008/FZA/14 was sought from Dental Research Ethics Committee for collecting of human teeth from the Tissue Bank (Leeds Dental Institute).

4.4.2 Experimental protocol

The same procedures were followed as described previously for bovine specimens.

1. There were five experimental treatment groups. Each experimental group was comprised of 10 human enamel slabs, obtained from 10 teeth.
2. Each experimental group was comprised of 10 enamel specimens. Each enamel slab was identified and given three figures, the letter H which was referred to human enamel and two numbers (the number of the tooth and the slab number) e.g. (H11), so each enamel slab had a relevant identification figure for testing (Appendix 2).
3. The series of slabs from each tooth were arranged in to resin blocks.
4. The enamel slabs were randomly assigned to one of the following groups:

4.1 Group 1(E1) Meridol®

4.2 Group 2(E2) Elmex® *anti-caries*

4.3 Group 3 (E3) Sensodyne Pronamel®

4.4 Group 4 (E4) Elmex® Sensitive Plus

4.5 Group 5 (E5) Aronal® (Control)

5. Each experimental group consisted of one acrylic plate with holes which was used to hold 10 enamel slabs. Slab preparations and baseline measurements for MHS (Appendix 2 in Table 8.2) and checking the flatness of the enamel slabs by profilometry scanning as in sections 3.3.3.4 and 3.3.3.5.
6. Randomisation: Same as section 3.3.3.9 (using 10 human premolars).
7. Blindness: same as in section 3.3.3.10
8. Reproducibility: same as in section 3.3.3.11
9. All groups underwent acidic challenge for 2 minutes in 0.3% citric acid (pH 3.6) at five intervals daily and subjected to twice/day tooth brushing for 2 min toothpaste / artificial saliva slurries application.
10. Profilometric scanning was performed after 7 days.
11. The same cycling procedure was repeated after 14 days, 21 days and 28 days.

Statistical advice was sought and the sample size was calculated by using software for power and sample size calculation in the IBM SPSS package of University of Leeds computer system. Assuming use of repeated measures Analysis of Variance for analysing the data with a standard deviation in control (3.03) from a previous *in vitro* study, minimum difference to detect 2 (effect size 0.66), intra-cluster correlation (0.981) and power of calculation would be 90% with an alpha = 0.5% and 10 samples per group were considered acceptable .

4.5 Results of study (part II)

4.5.1 Profilometric analysis

Some examples of the profilometric scans are displayed in (Appendix 7).

Using a cyclic model of erosive challenge with 0.3% citric acid (pH 3.6) for 2 mins x 5 times per days and tooth brushing abrasion for 2 mins x 2 times per day showed considerable enamel wear depth (µm) in relation to borh reference areas in the human enamel treated with Meridol® (AmF/SnF2, 1400 ppm F) (Figures 8.12 and 8.13). Less Enamel erosive / abrasive wear depths (µm) were found in enamel slabs treated with Elmex® *anti-caries* (AmF, 1400 ppm F) as shown in Figures 8.14 and 8.15, Sensodyne® Pronamel (NaF, 1450 ppm F) as shown in Figures 8.16 and 8.17. Elmex® Sensitive Plus (AmF, 1400 ppm F) as shown in Figures 8.18 and 8.19 and Figures 8.10 and 8.11 show some examples of enamel slabs treated with 0 ppm F toothpaste causing more enamel wear depth in comparison to enamel samples treated with Elmex® groups and Sensodyne® Pronamel toothpastes.

4.5.2 Data analysis of (study part II)

4.5.2.1 The distribution of data of the enamel surface loss changes from baseline

Normal distribution of the data was checked using the Kolmogrov-Smirov test. All the data were normally distributed for all groups and at the end of each experimental procedure after 7, 14, 21, 28 days (Appendix 8, Tables 8.11-8.14).

4.5.2.2 The effect of five different toothpastes treatments on human enamel surface loss (µm) subjected to 0.3% citric acid erosion and tooth brushing abrasion during experimental periods at 7,14, 21 and 28 days.

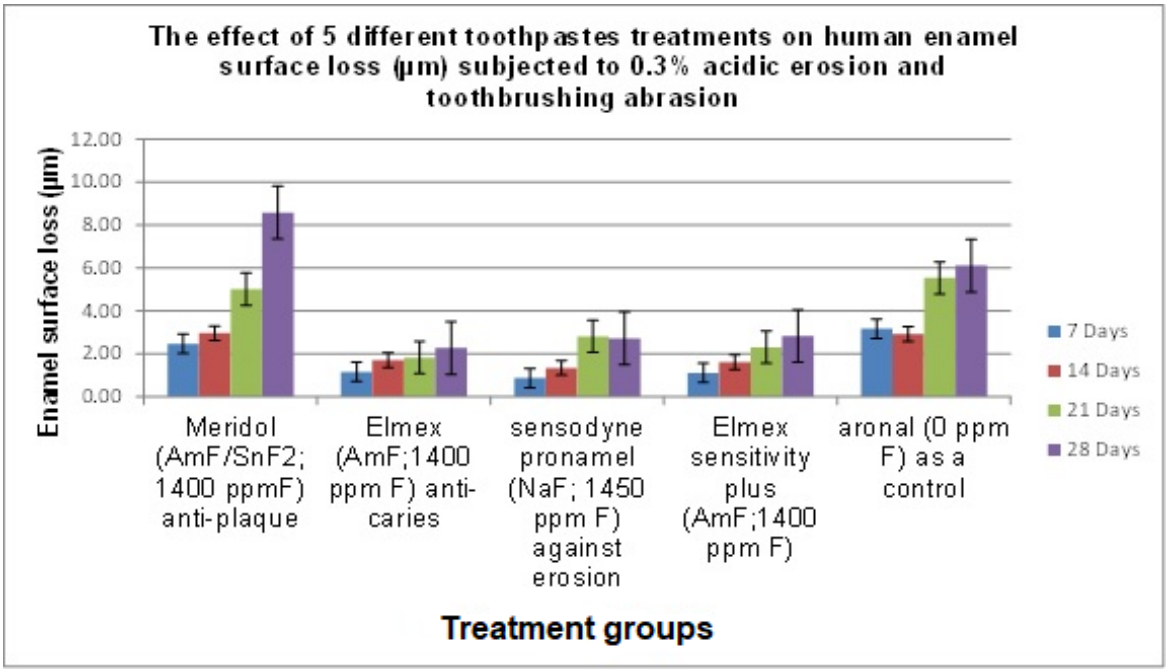


Figure 4.1 The effect of five different test toothpastes treatments on human enamel surface loss (µm) subjected to citric acid erosion and tooth brushing abrasion during the cycling procedures at 7, 14, 21 and 28 days.

Figure 4.1 shows a similar pattern of enamel surface loss (µm) that was previously seen for bovine enamel. All groups showed enamel surface loss (µm) which increased with continued cycling for 7, 14, 21 and 28 days compared to baseline. The most ESL (µm) was seen for the Meridol® (AmF/SnF) group, and then Aronal® (F-Free). Both Elmex® groups containing AmF, 1400 ppm F (Elmex® *anti-caries* and Elmex® Sensitive Plus) in addition to Sensodyne® Pronamel toothpaste (NaF, 1450 ppm F) showed less reduction in human enamel surface wear compared to Meridol® toothpaste (AmF/SnF2, 1400 ppm F) and Aronal® toothpaste (0 ppm F) after 7, 14, 21 and 28days of cycling procedures.

4.5.2.3 Changes of human enamel surface loss (µm) from baseline achieved by the five different toothpaste treatments twice daily under erosive challenge using 0.3% citric acid for two minutes five times daily.

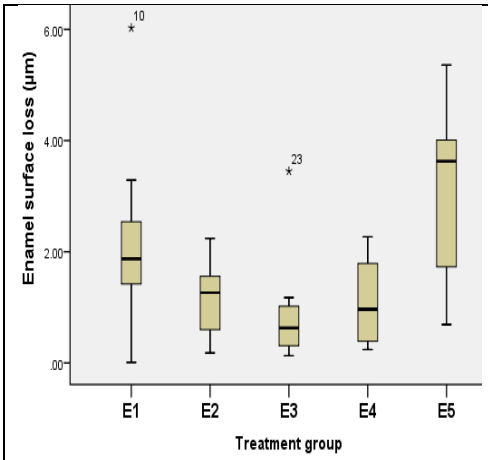


Figure 4.2 Changes in human enamel surface loss (µm) after 7 days

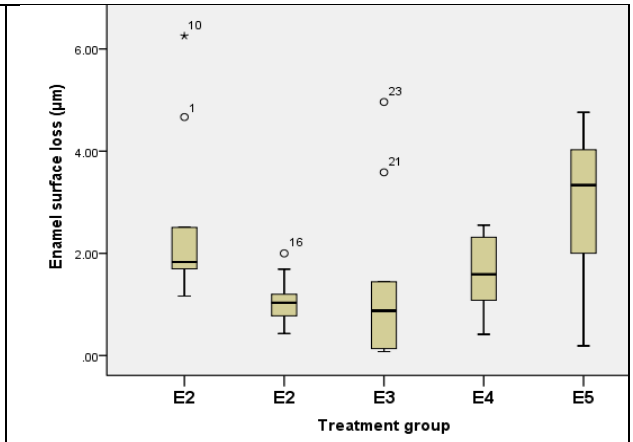


Figure 4.3 Changes in enamel surface loss after 14 days

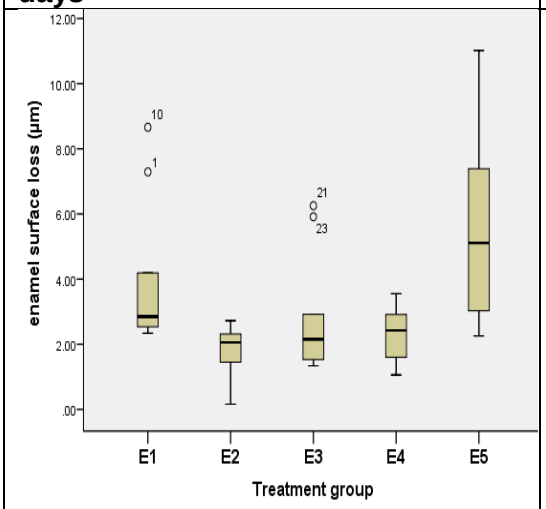


Figure 4.4 Changes in enamel surface loss after 21 days

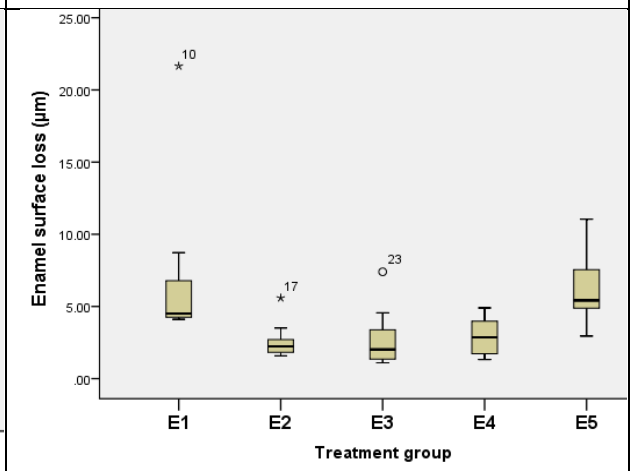


Figure 4.5 Changes in human enamel surface loss changes after 28 days

° and * are outliers and extreme values

Median changes, lower and upper quartiles of all groups after 7, 14, 21 and 28 days of erosion/abrasion for human enamel are shown in Figures 4.2-4.5. Elmex groups AmF, 1400 ppm F (Elmex® *anti-caries* and Elmex® Sensitive Plus) and Pronamel® continued to show similar results of median changes, whereas there was a greater difference for the Meridol® and Aronal® groups.

4.5.2.4 Distribution of human enamel surface loss (µm) changes during the experimental procedures

Table 4.1 shows the distribution of the mean enamel surface loss values (µm) mean, standard deviation, median, minimum and maximum amounts for each experimental group during the whole experimental period in the study and profilometry measurements were taken for each sample within the group at the end of 7, 14, 21, and 28 days.

Table 4.1: Distribution of human enamel surface loss (µm) changes achieved by 0.3% citric acid erosion and tooth brushing abrasion during experimental period procedures for all experimental groups after days 7, 14, 21, 28 days.

Duration	Toothpaste test Groups	Mean (µm)	Std. Dev.	Med.	Inter-quartile	Range	Min	Max.	95% Confidence Interval for Mean	
									Lower Bound	Upper Bound
7 Days	Meridol®	2.23	1.77	1.87	1.69	6.02	.01	6.03	.93	3.54
	Elmex® *anti-caries*	1.16	0.63	1.27	1.03	2.06	.18	2.24	.71	1.61
	Pronamel®	.87	.98	.63	.79	3.32	.13	3.45	.17	1.57
	Elmex® sensitive plus	1.12	0.78	.97	1.53	2.03	.24	2.27	.56	1.67
	Aronal®	3.17	1.57	3.63	2.66	4.67	.69	5.36	2.05	4.29
14 Days	Meridol	2.59	1.72	1.83	2.03	5.09	1.2	6.26	1.27	3.91
	Elmex® *anti-caries*	1.28	.76	1.04	.62	1.57	.43	2.00	.71	1.41
	Pronamel	1.35	1.65	.88	1.85	4.96	.08	4.96	.17	2.53
	Elmex® sensitive plus	1.62	0.73	1.60	1.32	2.14	.41	2.55	1.10	2.14
	Aronal®	2.92	1.49	3.34	2.36	4.57	.19	4.76	1.85	4.0
21 Days	Meridol®	4.00	2.34	2.89	3.26	6.32	2.33	8.66	2.20	5.80
	Elmex® *anti-caries*	1.83	0.76	2.06	.99	2.56	.76	2.72	1.28	2.37
	Pronamel	2.81	1.80	2.22	3.16	4.92	1.33	6.25	1.52	4.10
	Elmex® sensitive plus	2.31	.79	2.43	1.41	2.50	1.06	3.56	1.75	2.87
	Aronal®	5.54	3.04	5.11	5.02	8.77	2.25	11.0	3.36	7.72
28 Days	Meridol®	8.58	7.26	4.51	3.57	17.6	4.11	21.7	2.65	11.4
	Elmex® *anti-caries*	2.27	0.69	2.24	1.11	4.02	1.57	5.59	1.73	3.44
	Pronamel	2.72	1.97	2.02	2.35	6.30	1.10	7.40	1.32	4.13
	Elmex® sensitive plus	2.83	1.24	2.87	2.32	3.58	1.32	4.90	1.94	3.71
	Aronal®	6.11	2.39	5.42	3.14	8.10	2.95	11.0	4.40	7.81

4.5.2.5 ANOVA results for human enamel surface loss (µm) (HESL)

Table 4.2 Shows that using ANOVA test significant values were observed for all groups and at different periods of experimental procedures ($p<0.05$) which indicated that there were treatment effects between the groups, so the null hypothesis was rejected.

Table 4.2 Enamel surface loss (µm) significant changes between the treatment groups using ANOVA tests at all experimental periods at 7, 14, 21 and 28 days.

Duration			Sum of Squares	df	Mean Square	Sig.
Human ESL(µm) at 7 days	Between Groups	(Combined)	40.47	4	10.12	.000
	Within Groups		67.96	45	1.51	
Human ESL(µm) at 14 days	Between Groups	(Combined)	31.98	4	8.00	.006
	Within Groups		87.12	45	1.94	
Human ESL(µm) at 21 days	Between Groups	(Combined)	88.39	4	22.10	.001
	Within Groups		167.42	44	3.81	
Human ESL(µm) at 28 days	Between Groups	(Combined)	288.04	4	72.01	.001
	Within Groups		587.84	45	13.06	

Table 4.3 shows that Elmex® *anti- caries*, Elmex® Sensitive Plus and Pronamel® groups showed lower values for wear at p≤0.05 compared to Meridol® and Aronal® which was statistically significant.

Table 4.3 comparison between the five toothpastes on human enamel surface loss (µm) achieved by erosive challenge using 0.3% citric acid and tooth brushing abrasion from baseline until 7 days.

Pairwise comparisons		Mean Difference (µm)	Std. Error	p value	95% Confidence Interval	
					Lower Bound	Upper Bound
Meridol® E1	Elmex® *anti-caries*	1.76*	0.75	.02	0.25	3.26
	Elmex® Sensitive plus	1.96*	0.75	.01	0.45	3.46
Elmex® *anti-caries* E2	Pronamel®	0.08	0.75	.91	-1.59	1.42
	Aronal® F-Free	1.85*	0.75	.02	-3.36	-0.35
Pronamel® E3	Meridol®	1.67*	0.75	.03	0.170	3.18
Elmex® Sensitive Plus E4	Elmex® *anti-caries*	0.20	0.75	.79	-1.70	1.30
	Pronamel	0.28	0.75	.71	-1.79	1.22
	Aronal® F-Free	2.1*	0.75	.01	-3.56	-0.55
Aronal® F-Free E5	Pronamel®	1.77*	0.75	.02	0.27	3.27
	Elmex® Sensitive plus	2.05*	0.75	.01	0.55	3.56

* Mean value significant at the 0.05 level.

Table 4.4 shows that after 14 days of cycling, there were significant differences between the groups (p≤.01). Both Elmex® groups and Pronamel® treatments had a significantly lower ESL (µm) than Meridol® and Aronal®.

Table 4.4 Comparison between the five toothpastes on human enamel surface loss (µm) achieved by erosive challenge using 0.3% citric acid and tooth brushing abrasion from baseline until 14 days.

Pairwise comparisons		Mean Difference ((µm)	Std. Error	p value	95% Confidence Interval	
Meridol® E1	Elmex® *anti-caries*	1.90*	0.67	.007	0.55	3.24
	Pronamel®	1.79*	0.67	.010	0.44	3.13
	Aronal® F-Free	0.26	0.67	.699	-1.08	1.60
Elmex® *anti-caries* E2	Elmex® Sensitive plus	0.493	0.67	.464	-1.84	0.85
	Aronal® F-Free	1.64*	0.67	.018	-2.98	-0.29
Pronamel® E3	Elmex®	0.11	0.67	.871	-1.23	1.45
	Elmex® Sensitive plus	0.384	0.67	.568	-1.73	0.96
	Aronal® F-Free	1.53*	0.67	.027	-2.87	0.18
Elmex® Sensitive Plus E4	Meridol®	1.40*	0.67	.041	0.06	2.75
Aronal® F-Free E5	Elmex® *anti-caries*	1.64*	0.67	.018	0.29	2.98
	Pronamel®	1.53*	0.67	.027	0.18	2.87
	Elmex® Sensitive	1.14	0.67	.093	0.20	2.49

* Mean value significant at the 0.05 level.

Table 4.5 shows the multiple comparisons between the groups showing significant mean change differences (µm) with Elmex® *anti-caries*, Elmex® Sensitive plus and Sensodyne Pronamel® compared to Meridol® (p≤0.005) and no significance between Meridol® and Aronal® (p>0.05). The mean difference (µm) for Aronal® with the other treatment groups was significant (p≤0.005).

Table 4.5 shows comparisons between the five toothpastes on human enamel surface loss (µm) achieved by erosive challenge using 0.3% citric acid and tooth brushing abrasion from baseline until 21 days.

Comparison between treatment groups		Mean Difference (µm)	Std. Error	p value	95% Confidence Interval	
					Upper Bound	Lower Bound
Meridol® E1	Elmex® *anti-caries*	3.34*	1.17	.006	.986	5.69
	Pronamel®	2.24	1.17	.06	-0.12	4.59
	Elmex® Sensitive	2.99*	1.17	.01	.63	5.34
	Aronal® F-Free	.20	1.17	.86	-2.56	2.15
Elmex® *anti-caries* E2	Pronamel®	1.10	1.17	.35	-3.46	1.25
	Elmex® Sensitive	0.35	1.17	.76	-2.71	2.00
Pronamel® E3	Elmex® *anti-caries*	1.10	1.17	.35	-1.25	3.46
	Elmex® Sensitive	0.75	1.17	.53	-1.61	3.10
Elmex® Sensitive E4	Elmex® *anti-caries*	0.35	1.17	.76	-2.00	2.71
Aronal® F-Free E5	Elmex® *anti-caries*	3.54*	1.17	.004	1.19	5.90
	Pronamel®	2.44*	1.17	.04	.09	4.80
	Elmex® Sensitive	3.19*	1.17	.01	.835	5.54

* Mean value significant at the 0.05 level.

Table 4.6: Data analysis at day 28

At the end of cycling procedure (after 28 days), statistical analysis showed Elmex® *anti-caries*, Elmex® sensitive Plus and Pronamel® had lower values for surface loss ($p<0.005$), whereas Meridol® and Aronal® showed higher values for surface loss (µm). No significance between Meridol® and Aronal® ($p=0.93$ Table 4.6).

Table 4.6 comparisons between the five toothpastes on human enamel surface loss (µm) achieved by erosive challenge using 0.3% citric acid and tooth brushing abrasion from baseline until 28 days.

Comparison between the groups		Mean Difference (µm)	Std. Error	p value	95% Confidence Interval	
					Lower Bound	Upper Bound
Meridol® E1	Elmex® *anti-caries*	6.60*	1.69	.000	3.187	10.01
	Pronamel®	5.72*	1.69	.002	2.30	9.13
	Elmex® Sensitive	6.03*	1.69	.001	2.62	9.44
	Aronal®	2.92	1.69	.09	-.49	6.33
Elmex® anti-caries E2	Pronamel®	0.88	1.69	.61	-4.29	2.53
	Aronal® F-Free	-3.68*	1.69	.04	-7.09	-.265
Pronamel® E3	Elmex® *anti-caries*	0.88	1.69	.61	-2.53	4.29
	Elmex® Sensitive	0.32	1.693	.85	-3.09	3.73
Elmex® Sensitive E4	Elmex® *anti-caries*	0.56	1.69	.74	-2.85	3.97
	Pronamel®	0.32	1.69	.85	-3.73	3.09
	Aronal® F-Free	3.11	1.69	.07	-6.52	.299
Aronal® (F-Free) E5	Pronamel®	2.79	1.69	.01	-0.618	6.20
	Elmex® Sensitive	3.11	1.69	.01	-0.299	6.52

* Mean value significant at the 0.05 level.

4.5.2.6 Comparison of tooth surface loss (µm) between bovine and human enamel

Figures 3.8 and 4.1 show similar trends towards the effect of 0.3% citric acid erosion and tooth brushing abrasion on both bovine and human enamel treated with the five test toothpastes. It was shown that most ESL (µm) was seen in the Meridol® (AmF/SnF) group followed by the Aronal® (0 ppm F) group. Both Elmex® groups (Elmex® *anti-caries* and Elmex® Sensitive Plus), containing AmF, 1400 ppm F, Sensodyne® Pronamel toothpaste (NaF, 1450 ppm F) performed similarly in showing less reduction in ESL (µm) during the repeated cycling procedures after 7, 14, 21 and 28 days.

Tables 3.11 and 4.6 showed at the end of cycling procedure (after 28 days) that statistical analysis for both bovine and human enamel showed similar trends in the levels of enamel surface loss (µm) changes from baseline among the groups (Elmex® *anti-caries* (AmF; 0.14%), Elmex® Sensitive Plus (AmF; 0.14% F) and Sensodyne Pronamel® (NaF; 0.15% F+ 5% w/w KNO_3) had lower values for surface loss ($p<0.005$), whereas the Meridol® (AmF/SnF^2) and Aronal® groups had higher values for surface loss. There was no significance between Meridol® and Aronal®.

Two independent - t-test samples were conducted to compare the bovine ESL and Human ESL after 7, 14, 21 and 28 days which showed that there were no statistically significant differences ($p > 0.05$) between the bovine and human enamel surface loss changes.

After 7 days both enamel types showed no differences (mean =-.12, Std. deviation = 0.91, (t) = -.29 and p=0.78; after 14 days showed no differences mean = 0.38, Std. deviation = 0.92 , (t) = 0.93 and p = 0 .41; after 21 days also

presented no statistical differences at p= 0.41 (Mean ± Std. deviation) (0.43 ± 1.04), (t) = 0.92; and after 28 days, both enamel types demonstrated no statistical differences at p=0.90 with the mean ± Std.deviation = (0.06 ± 1.02) and (t) = 1.36.

4.6 Discussions of study 1 (parts I and II)

Pathological tooth wear is a well-known problem in clinical dental practice (Amaechi and Higham, 2005). The leading factor in dental tissue wear is the interplay between erosion of tooth hard tissues by exogenous or endogenous acids and intra-oral abrasive forces caused by tooth brushing. Reasons for using bovine enamel specimens in this study have been discussed recently and these enamel sources represent an acceptable substitute for human dental hard tissue.

4.6.1 Design of the study

4.6.1.1 De/remineralisation procedures

In this study demineralisation and remineralisation procedures were used to evaluate the effects of the different commercially used dentifrices. Citric acid cycling models including demineralisation periods might possibly try to simulate the dynamics of the clinical situation more adequately (five times consumption of acidic food and drinks each for two minutes duration). Other studies have induced in vitro eroded dental specimens by immersion of the dental samples into the erosive solution for longer periods ranging from 20 min immersion in 5 ml 1% citric acid (Burgmaier et al., 2002), immersion in 0.05 M citric acid for 3 h (Ganss et al., 2000), exposure to 0.3% citric acid (pH 3.2) for 30 min, 1, 2, 3 and 4 hours (Eisenburger et al., 2000), 1 h immersion in orange juice (Amaechi and Higham, 2001). These exaggerated erosive immersion times might be produced in certain dietary habits like drinking fruit juices in nursing bottles particularly during bed time (Al-Majed et al., 2002) or in some chronic diseases in which the erosive acid comes in contact with the teeth e.g. GORD

(Sivasitamparam et al., 2002), Remineralisation procedures achieved by artificial day time saliva and topical applications of toothpaste slurries for two minutes during tooth brushing procedures enables the opportunity to effectively monitor erosive-prevention and treatment regimens on dental hard tissues on a short time basis simulating the everyday scenario.

4.6.1.2 Method of analysis

In the present study, the determination of the enamel loss was chosen via profilometry because it is widely used in both in vitro and in situ studies (Jager et al., 2008, Rios et al., 2008b) and was considered as a method of choice for measuring the loss of the surface layer because there was no physical contact between them, so no damage will occur on the softened surfaces of dental hard tissue (Barbour et al., 2003). It may demonstrate an accurate, fast, simple and reproducible method over a reasonable area of dental tissue. The main disadvantage is that the samples need to be ground flat before scanning (Barbour and Rees, 2004).

4.6.1.3 Discussion of the enamel type

With the aim to verify whether to use bovine enamel as a substitute for human enamel in future dental erosion research in the present study, the in vitro assessment of the potential effects of the application of different specialised fluoridated toothpastes on the treatment and prevention of 0.3% citric acid erosive wear and tooth brush abrasion were performed for both bovine and human enamel under the same controlled laboratory procedures.

The present study showed bovine and human enamel both demonstrated similar trends towards 0.3% citric acid erosion and twice daily tooth brushing

abrasions which are in agreement with other studies (Meurman and Frank, 1991).

However, another study showed that bovine permanent enamel had twice the erosion compared to human permanent enamel when subjected to orange juice for 5 mins / 6 times daily (Amaechi et al., 1999a). Furthermore, the age of the bovine teeth plays an important role in the similarity of features of the radiodensity and hardness of bovine and human enamel as reported by Fonseca and co-workers (Fonseca et al., 2004, Fonseca et al., 2008). In a comparative analysis of bovine and human teeth for radiographic density of enamel and dentine, it was found that bovine enamel was slightly more mineralised than human enamel and that this could be attributed to the dietary habits and age of the herd from which the teeth were collected (Tanaka et al., 2008). The sources of human and bovine dental enamel in this study were carefully selected e.g. the human enamel slabs were obtained from freshly extracted premolars (for orthodontic reasons from young people) and similarly, the lower incisors of bovine teeth were collected from young cattle that were used for meat consumption and were in addition to the controlled and standardised in similar living conditions. In an *in vitro* study (Imfeld, 2001a) no differences were found following mechanical tooth brushing when applied to bovine and human dental tissues under the same standard procedures.

4.6.1.4 Discussion of the results of the experimental products

4.6.1.4.1 Treatment groups description

A group of five specialised toothpastes were selected according to their individual formulae which were specifically formulated to target different specific

oral diseases as mentioned by the manufacturers with the aim to test them for anti-erosive and tooth brushing abrasion resistance on enamel. They were comprised of four fluoridated toothpastes which contained different delivery systems of fluoride and similar concentrations. These were: Meriodol® (SnF/AmF; 1400 ppm F was specified for gingival regeneration (anti-gingivitis) and grouped as E1); Elmex® Sensitive plus (AmF ; 1400 ppm F was claimed as an anti-sensitivity paste and grouped as E2); Elmex® anti-caries (AmF; 1400 ppm F for caries protection grouped as E3); Sensodyne Pronamel® (NaF; 1450 ppm F and was claimed as daily protection against erosion as group E4 and then Aronal® which was considered as a control group (0 ppm F) grouped as E5 and was also formulated as a medicated toothpaste for inflamed gums and for cleaning teeth and massaging gums. So all the experimental toothpastes in this study had specific treatment purposes including the control toothpaste and in addition most of them except the control had similar fluoride concentrations (ppm F). Furthermore, three toothpastes Elmex® Sensitive Plus, Elmex® *anti-caries* and Meridol® had similar ppm F concentrations; in contrast to Sensodyne Pronamel® toothpaste with a fluoride concentration of 1450 ppm F and an advanced anti-dental erosion formula plus 5 % potassium nitrate (KNO_3). These unique and careful selections of the experimental groups make this study original.

4.6.1.4.2 Results of amine groups (AmF; 1400 ppm F)

The present study results revealed that the treatment of enamel slabs for both bovine and human teeth with (Elmex® Sensitive Plus, AmF,1400 ppm F) showed the lowest erosive / abrasive enamel surface loss values (µm) (mean ±

standard deviation) during the whole experimental period under the erosive /abrasive model compared to Meridol® (anti-gingivitis toothpaste) and with the fluoride-free toothpaste (control toothpaste).

The present study results showed a marked statistical significance ($p \leq 0.001$) that was obtained from the specialised fluoridated toothpastes (with special formula) (Elmex® *anti-caries* protection containing AmF, 1400 ppm F) compared to SnF_2 / AmF (Meridol®) toothpaste. This aimed at preventing new plaque deposition and promoting gingival health, with the added comparison to the fluoride-free toothpaste control ($p \leq 0.01$) under the present study procedures (repeated acidic attacks for 2 minutes/ 5 times per day and twice daily application of the amine products).

It has been reported that amine fluoride in the form of Elmex® rinsing solution containing 100 ppm F as Olaflur applied after enamel softening, abrasion decreased from 0.25 to 0.20 µm compared to rinsing with Dectaflur amine fluoride solution which did not show any decrease in abrasion (Lussi et al., 2004a). Enamel Loss by subsequent toothbrush abrasion of softened human enamel (0.28 µm) was up to 10 times greater than that of enamel without an erosive pre-treatment (Lussi et al., 2004a). Toothpastes containing a NaF form calcium-like material used on enamel surfaces (Cruz et al., 1992), could be enhanced by increasing the time of exposure and decreasing the pH, concentration and calcium availability but the time of exposure is the major factor (Saxegaard and Rölla, 1988, Cruz et al., 1992). An *in vitro* study (Brighenti et al., 2006) found that a low-fluoride toothpaste (550-µg F/g) with low pH (5.5) had the same anti-cariogenic action similar to the neutral product. In the present study Elmex® *anti-caries* (1400 ppm amine fluoride) and Elmex®

Sensitive Plus (1400 ppm F) toothpaste groups that contained amine fluoride and artificial saliva formulations were applied topically twice / a day with a tooth brushing procedure using 15 strokes with 300 g loads showed significant low enamel surface loss. Amine fluorides were introduced by GABA International in 1950s and the most commonly used amine fluoride product is Olaflur (N-Octadecyltrimethylendiamine-N.N.N-tris (2-ethanol)-dihydrofluoride which is used in Elmex products that act like surfactants, reducing the surface tension of saliva, and forming a homogeneous layer on all oral surfaces (GABA, 2009)[2]. It was thought that amine fluoride covered the tooth surfaces with a homogeneous molecular layer to form a continuous film that prevented rapid rinsing off by saliva and amine fluoride is considered to be available as an active agent for a longer period. Amine fluorides have a slightly acidic pH, and for these reasons fluoride ions can combine rapidly with calcium in dental enamel to form a calcium fluoride layer.

The effect of fluoride compounds in the form of AmF/NaF (250 ppm F, pH 4.3) and AmF/SnF_2 (250 ppm F, 390 ppm Sn; pH 4.2) solutions on enamel erosion under de/remineralisation procedures for 10 days, citric acid (0.05 M, pH 2.3) 6x2 min daily followed by treatment solution 6x2 min, mineral content was monitored by LMRG and SEM evaluation, and mineral loss reduction was found in AmF/SnF_2 solution treatments (Ganss et al., 2008). Similarly in the present study AmF/SnF_2 toothpaste (Meridol®) showed the most erosive level whereas AmF toothpastes and NaF (Pronamel) showed a superior significant level compared to the AmF/ SnF_2 and control groups. Pronamel® toothpaste was

[2] GABA International AG (2009), Dental professionals/ active substances, amine fluoride. http://www.gaba.com/htm/417/en/Amine-fluorides.htm?Subnav=&Subnav2=AmineFluoride&Article=16850

found to give only 48% protection against erosive challenge with 0.2% citric acid for 1hr (Rees et al., 2007). Nevertheless, the use of intensive fluoridation products was suggested and demonstrated by (Ganss et al., 2001b).

The results of this study demonstrated that there was a significantly smaller enamel surface loss (µm) in bovine and human enamel specimens that were treated with both Elmex® *anti-caries* (AmF, 1400 ppm F), Elmex® Sensitive Plus (AmF, 1400 ppm F) and Pronamel® (NaF, 1450 ppm F) compared to Meridol® (SnF_2/AmF) and Aronal® (control, fluoride-free toothpaste) with extended periods of acidic challenge using 0.3% citric acid for 5 times/a day and tooth brusing abrasion during application of fluoridated or non-fluoridated toothpastes. But there was a slight favour for Elmex toothpaste over Pronamel toothpaste which was not statistically significant. This finding was similar to that reported in an earlier study (ten Cate et al., 2008).

Interestingly, Elmex® *anti-caries* which has a similar fluoride source and concentration of Elmex® Sensitive Plus (AmF, 1400 ppm F) demonstrated the lowest minimum values of enamel surface loss (µm) and showed similar trends as Elmex® Sensitive Plus and Sensodyne® Pronamel against erosion. The ideal effect of toothpaste should be able to protect against caries and erosion, but the toothpaste with anti-caries bioavailability is not always able to protect against erosion even if it is of high fluoride concentration (Rios et al., 2008b, Moretto et al., 2010).

An *in vitro* study (Ganss et al., 2011a) concluded that some new special anti-erosion formulations were not effective regarding erosion protection and tooth brushing prevention. The investigators performed two experiments on human

enamel specimens testing 8 conventional toothpastes with NaF (from 1400 to 1490 ppm F), there were four with anti-erosion formulations (two fluoride toothpastes, one contained NaF + KNO_3 and the other had NaF + 1% hydroxyapatite formula; two experimental anti-erosion toothpastes which were fluoride-free and had zinc-carbonate-hydroxyapatite (biorepair was fluoride free formula), and the other one had chitosan, (chitodent no fluoride); and two Sn-containing products (toothpaste: 3,436 ppm Sn, 1,450 ppm F as SnF_2 / NaF; gel: 970 ppm F, 3,030 ppm Sn as SnF_2)n. A mouthrinse named Elmex® erosion contained 500 ppm F as AmF/NaF, 800 ppm Sn as $SnCl_2$) was the positive control while the negative control was Aronal® (fluoride-free toothpaste). In experiment 1, enamel samples were exposed to acidic erosion with citric acid, pH 2.4, 6 × 2 mins/day for 10 days, and toothpaste slurries were applied (2 × 2 mins/day) and intermittently stored in a mineral salt solution. Additionally, in experiment 2**,** samples were brushed for 15 s during the slurry application.

The time between the cycles was 1.5 h, the measurement of enamel loss was recorded profilometrically. In erosion experiment 1, enamel loss was found in the products ranging from (19 to 78%) compared to the negative control, but with the positive control all products were less effective against erosion. It was found that the gel and most NaF toothpastes and one of F-free formulation reduced tissue loss significantly (between 19 and 42%). The Sn-containing formulations were the most effective (toothpaste and gel showed 55 and 78% reduction, respectively). In experiment 2, only 4 NaF toothpastes (Pronamel (GlaxoSmithKline) with KNO3, 1,450 ppm F as NaF was most effective in reducing dental tissue loss, then Theramed 2 in 1 Original 1,450 ppm F as NaF, GUM Original Whit (Sunstar 1,490 ppm F as NaF, Perlodent Kraeuter 1,450

ppm F as NaF) revealed significant effects compared to the F-free control (reduction between 29 and 37%). The F-free special toothpastes and the Sn toothpaste had no significant effect The Sn gel had a 75% reduction and revealed the best result compared to conventional NaF toothpastes reduced the erosive tissue loss with limited efficacy regarding the prevention of brushing abrasion. The special formulations were not found to be superior, or were even less effective. Comparisons between the NaF toothpastes revealed only minor differences. F-free products such as BioRepair (no fluoride anti-erosion toothpaste) showed no significant effect compared to the negative control and Chitodent that contained chitosan and also had no fluoride reduced the enamel loss by up to 30% compared to conventional NaF products. Compared to the present study, the test products contained different specialised formulations with specific properties and nearly similar concentrations of fluoride. They were tested against the repeated erosive exposure to 0.3% citric acid and twice daily tooth brushing abrasion with an incubation period of 1 hr between the dippings. However, the tested products demonstrated different levels of effectiveness against erosive and abrasive wear, but the toothpaste (1450 ppm NaF + 5% that were anti-erosion formulations and in addition to Elmex *anti-caries* showed the greatest protection against enamel wear compared to fluoride-free and AmF/SnF_2 toothpastes.

4.6.1.4.3 Sensodyne Pronamel® (1450 ppm F of sodium fluoride; NaF toothpaste and 5% potassium nitrate)

Sensodyne Pronamel® toothpaste contains 1450 ppm F of NaF was introduced by GlaxoSmithKline Consumer Healthcare (GSK) especially to help protect against daily acidic erosion in addition to cleaning properties. The addition of 5%

w/w of potassium nitrate (KNO_3) to the ingredients provides treatment of dentine hypersensitivity.

The results of the present study proved that Sensodyne Pronamel® toothpaste showed remarkably significant effects on mechanical tooth brushing abrasion and 0.3% citric acid erosion during periodic treatment for four weeks (at 7, 14, 21, 28 days). These effects might be related to the bioavailability of fluoride in these recently developed toothpastes (Hara et al., 2009b) that demonstrated the influence of fluoride availability of similar sources and concentrations (1,450 ppm NaF and 1,450 ppm NaF+ 5% KNO_3) on eroded bovine enamel and found different levels of remineralisation on eroded enamel. In contrast, the *in vitro* investigation of Sensodyne Pronamel (1450 ppm F) with two different dentifrices Sensodyne Cool Gel (1100 ppm F), and another highly concentrated fluoride toothpaste PrevDent 5000 (5000 ppm F) with the control being de-ionised water to verify the capability of controlling enamel erosion progression caused by hydrochloric acid HCl. solution (pH 1.2; 0.1M). This was assessed in 60 bovine enamel slabs divided into 4 groups contained 15 specimens in each group were immersed in HCl for five cycles and stored in artificial saliva for 1 h, the slabs were exposed to different test dentifrices.

The study demonstrated that the erosive damage that was caused by a simulated intrinsic erosive challenge (HCl acid) seemed unable to be controlled by fluoridated dentifrices, even in the presence of high concentrations fluoride ions (5000 ppm F) (Messias et al., 2011). The clinical effect of a NaF-containing toothpaste may thus well depend on an initial formation of alkali-soluble fluoride that may deposit onto the enamel crystals and inhibit further demineralisation or increase the rate of remineralisation. This was demonstrated in an *in vitro* experiment which was performed on human enamel

samples obtained from unerupted human molars in which, four different types of NaF-containing toothpastes (all contained about 1000 ppm F, pH 7 of the supernatants); (1) Colgate Junior, Colgate-Palmolive Co., USA; (2) Colgate Tandsten Kontrol, Colgate-Palmolive A/S, Denmark; (3) Crest Regular Flavour, Procter & Gamble, USA; (4) Gleem, Procter & Gamble, USA, both brands of Colgate-Palmolive also had Na-pyrophosphate as an ingredient. The four tested toothpastes in this experiment were applied by soaking standard size cotton pellets, for 1 and 24 h and it was found that all NaF-containing toothpastes that were applied topically over two different periods of time caused deposition of alkali-soluble fluoride (calcium fluoride-like material and adsorbed fluoride) on the enamel surfaces treated with different toothpaste supernatants and increased with the time exposure (Cruz et al., 1992).

Perhaps the results may be overestimated as they used unerupted third molars in which their crowns were still immature and not fully remineralised.

In addition to the toothpaste, application was achieved using the soaked cotton pellets for a longer period of exposure for one and 24 hours reflecting an unrealistic daily application of toothpaste with tooth brushing home care practices.

Similarly, in an *in vitro* model sodium fluoride dentifrices can increase the protection of eroded human enamel specimens during a daily cycling regimen involving five erosive challenges for two-minutes using 1% citric acid at pH 3.8, with three two-minute treatments (1:1150 ppm NaF, 2: Crest Cavity Protection 1100 ppm NaF, 3: Elmex Sensitive (1450 ppm amine F) and a fluoride-free placebo. The remineralisation was performed in a mixture of human saliva and mucin-containing artificial saliva. Measurements using surface microhardness

(SMH) were taken at baseline, 10, and 20 days. It was found that SMH for a 1450 ppm NaF test dentifrice was greater than for Elmex Sensitive (1450 ppm amine F) and placebo at 10 days, while both products were greater than the placebo at 20 days. The authors suggested that the increased protection of enamel *in vitro*, correlated with fluoride uptake (Newby et al., 2006). The most important aspect was the formulation effects of the oral products on driving the performance on the protection against daily tooth surface loss. In contrast to the present study, Elmex sensitive plus (1400 AmF) had a lower fluoride concentration than that used in the above study and produced less enamel loss. However, 1450 ppm NaF resulted in a similar reduced amount of enamel surface loss (µm) as in the present study.

4.6.1.4.4 Meridol® (amine and stannous fluoride; AmF/SnF2 toothpaste)

Amine fluoride (AmF, 350 ppm F) and stannous fluoride (SnF_2, 1050 ppm F) toothpaste was produced by GABA International AG (Basle/Switzerland) under the trade name Meridol®. It combines the antimicrobial effect of (SnF_2) and anti-caries effect of (AmF) and was aimed at reducing pathogenic microorganisms and reducing the clinical signs of gingivitis (Zimmermann et al., 1993, Mengel et al., 1996, Laine et al., 2005).

However, the products of the Meridol® gum protection system were formulated to inactivate residual plaque and inhibit the formation of new plaque. Maintaining healthy gums is simply by daily tooth cleaning to reduce bacterial plaque, inhibiting new plaque deposition and eliminating calculus formation Loe and Holm-Pedersen, 1965, (Lindhe et al., 1975, Zimmermann et al., 1993).

It is beneficial for providing effective control of gingivitis by reducing plaque accumulation through advising certain specialised chemico-pharmacological effective preparations in addition to regular mechanical tooth cleaning as it was revealed that the reduction in potentially gingival pathogenic bacteria was slightly higher in the amine/stannous fluoride group in a comparative clinical double-blind 9-month study. This was designed to examine the efficacy of amine/stannous fluoride (AmF/SnF_2) (Meridol®) and sodium fluoride (NaF) in 150 participants with chronic gingivitis or early signs of periodontitis that were divided into 3 randomised groups. Group1: Was given a NaF toothpaste and an NaF mouthrinse, group 2: AmF/SnF; toothpaste and mouthrinse, and group 3: AmF/SnF, toothpaste and NaF mouthrinse (Mengel et al., 1996).

In the present study AmF/SnF_2 toothpaste (Meridol®) showed the most erosive level whereas AmF toothpastes and NaF (Pronamel®) showed superior significant levels compared to AmF/SnF_2. Toothpaste having AmF/SnF_2 reduced mineral loss by 30% in a study by Ganss (2004) but continuously increasing significant mineral loss values were observed also after 5 days during an *in situ* study compared to the other days ($p \leq 0.001$) (Ganss et al., 2001). The possible explanation for the increase in mineral loss values could be because the product was specialised for anti-gingivitis and for that reason it could possibly had high efficient cleaning properties to counteract plaque and calculus deposition. AmF/SnF_2 toothpaste has inhibitory effects in dental caries by enhancing accumulation of F in plaque and is effective against gingivitis by decreasing the sulcus bleeding index values by 30% and 50% when using both Meridol toothpaste and Meridol mouthrinse (Bánóczy et al., 1989), by reducing acid metabolism in dental plaque and oral bacteria.

This antimicrobial action of the AmF/SnF_2 toothpaste was optimised when combined with Meridol mouth rinse which lasted for a longer period of over 6 hours intra-orally (Gerardu 2006). However, various oral hygiene products such as fluoride toothpastes and mouth rinses have a low pH to enhance the chemical stability of some fluoride agents and helps in the formation of fluorhydroxyapatite (e.g. in white spot lesions). Furthermore, the precipitation of calcium fluoride on the tooth surface acts as protection against acid attack (Fischer et al., 1995; ten Cate, 1997). A similar result was found that Meridol among with the other tested products exhibited no protection against erosion (Lussi et al., 2008). Meridol® (AmF/SnF_2) toothpaste contains hydrochloric acid in its ingredients and that may explain the high erosive values of the product. Similarly, it was found that in an *in vitro* study on bovine enamel one of the tested oral hygiene product had four times the erosive effect on bovine enamel which was more than the erosive sports drink owing to the presence of organic acids and some chelating substances in some oral hygiene products that can cause deleterious effects on teeth (Rytomaa et al., 1989). In contrast, the effect of fluoride compounds in the form of AmF/NaF (250 ppm F, pH 4.3) and AmF/SnF_2 (250 ppm F, 390 ppm Sn; pH 4.2) solutions on enamel erosion under de/remineralisation procedures for 10 days, citric acid (0.05 M, pH 2.3) 6x2 mins daily followed by treatment solution 6x2 mins. Mineral content was monitored by LMRG and SEM evaluation, mineral loss reduction was found in AmF/SnF_2 solution treatments (Ganss et al., 2008).

Meridol products with 1400 ppm F can be prescribed to individuals who are not able to achieve sufficient plaque removal by performing oral hygiene measures at home. They can then achieve proper tooth cleaning by choosing an agent with conventional fluoride source but with high cleaning efficiency and without

using highly concentrated fluoride products. However, in terms of plaque control, the combined use of an AmF/SnF_2 dentifrice and mouthrinse was found to be more effective in plaque control reduction compared with the use of NaF toothpaste and mouthrinse in a group of people suffering from periodontal disease (Paraskevas et al., 2004). Dental plaque was considered to protect the covering enamel against acidic attacks and thus inhibiting the dissolution of the dental tissues by preventing the direct acidic contact to those covering areas of the enamel (Sorvari, 1989).

4.6.1.4.5 Control group (fluoride-free toothpaste)

The control group (Aronal) in this study had no fluoride source contained a special formula of dicalcium phosphate dihydrate, allantoin and aluminium lactate and claimed by the manufacturer as a medicated agent for gums. Fluoride-free toothpastes may be prescribed for certain individuals who are sensitive to fluoride, advised to avoid its use, personal choice and also young children who are not able to spit properly. The control group had more enamel surface loss than the other test groups except for the Meridol group. In contrary to the present study, many studies have used de-ionised water as a control group (Kelly Edenbridge and Smith, 1988, Hara et al., 2009a).

4.6.1.4.6 The abrasive type in the experimental toothpastes

Generally, all the experimental toothpastes in the present study contained hydrated silica except the control test product which contained silica as an abrasive substance. Recently, in modern toothpastes there have been trends toward incorporation of hydrated silica and improving cleaning power of the toothpastes without increasing the RDA (relative dentine abrasivity) values evaluated the cleaning efficacy in comparison with abrasivity on dentine (RDA

value for 41 toothpastes available to European consumers in 1995) and for cleaning power assessment, a modified pellicle cleaning ratio (PCR) measurement method was developed in a five-day tea-staining procedure on bovine anterior tooth slabs (Wiilknitz, 1997).

The authors found that the majority of toothpastes (80%) had an RDA value below 100. Only three products exceeded the reference in cleaning power. Most products (73%) had a cleaning power (PCR value) between 20 and 80 (Wiilknitz, 1997).

Other substances may be added such as polishing alumina, showed improved cleaning power as in some active ingredients, especially sequestrants (stain remover substances) such as sodium tripolyphosphate or AHBP (Bisulphonates), also improved the PCR/RDA ratio by stain-dissolving action without being abrasive. There was also a general trend toward decreased abrasivity in the oral care products without loss of cleaning efficacy that could be noticed on the European toothpaste market mostly due to the increased use of high-performance abrasives such as hydrated silica (Wiilknitz, 1997).

Furthermore, it is known that not only can chemically different types of abrasives have different cleaning/abrasivity patterns but also chemically identical abrasives such as hydrated silica or calcium carbonate can differ distinctively in these effects and can also have different cleaning / abrasivity ratios. The mixture of chemically different abrasives can result in effects which differ distinctively from those of the individual components (Barbakow et al., 1987).

It is clearly apparent that not only is the abrasive relevant to wear of dentine but also the detergent both interacting to produce variable amounts of wear dependent on which combination is contained (Moore and Addy, 2005).

The presence of fluoride and the abrasive can modulate the effect of the development of surface loss due to abrasion and erosion lesions in enamel whereas the abrasivity of the dentifrices had the major impact on dentine surface (Hara et al., 2009a).

That study was conducted on eight experimental groups of human enamel and dentine slabs subjected to demineralisation in 1% citric acid solution (pH 3.73) for 2 minutes, then 60 minutes immersion in artificial saliva and brushing with the experimental dentifrices with low, medium, high abrasive levels as defined by REA/ RDA of dentifrices and fluoride 1100 ppm F (NaF) or no fluoride. One hundred ml of dentifrice and artificial saliva slurry (1:3, w/v) was used to brush the specimens and the control group was de-ionised water.

The demineralisation, remineralisation and tooth brushing were repeated 3 times a day for three days, then the surface loss was analysed by optical profilometry. The results of the study for presence of fluoride and no fluoride in dentifrices was significant for enamel $p < 0.05$ whereas the surface loss of dentine groups brushed with fluoride dentifrices were not different from non-fluoride groups except for the low abrasive formulations which were not significant in dentine surface specimens (Hara et al., 2009a).

However, that abrasion and erosion model is different from our methodological procedure in erosive and tooth brushing challenges. The study by (Hara et al., 2009a) have used 1% citric acid (pH 3.73) as an erosive solution for 2 minutes

exposure 3 times/day and brushing with one of the test groups for 3 days only, compared to the present study, immersion of the specimens in 0.3% citric acid solution (pH 3.6) for 2 minute / 5 times per day and brushing twice a day (morning and evening), then profilometric readings at 7, 14, 21 and 28 days representing a more realistic daily life oral practice.

The present study, demonstrated that all test groups showed progression in enamel surface loss with different effects after extended periods (28 days) of acidic challenge by 0.3% citric acid for 2min/5 times daily and tooth brushing abrasion during toothpaste application 2 min/2 times daily. The increasing of the initial erosive lesions on enamel or dentine surfaces that were characterised by tooth surface loss with the duration and the number of acid attacks was also noted in another study by (Meurman and Frank, 1991).

In a study by `(Lussi et al., 2008) examined three toothpastes that claimed to prevent erosion. They were compared with two conventional toothpastes and a positive and negative control. The tested toothpastes showed comparable but different fluoride content ranging from 1,100 to 1,450 ppm. None of the tested toothpastes showed statistically significant better protection than another against an erosive attack. In that study no overall better performance could be shown of the slightly acidic toothpaste slurries (Meridol®) compared to the neutral ones. The investigators also found that Meridol® toothpaste had no effect on erosion protection (Lussi et al., 2008), which is in agreement with the present study.

In contrast another study by (Hughes et al., 2004) tried to verify the protective effect of different fluoridated products on enamel against erosion attributed to citric acid and citric acid–based soft drinks such as, a RTD Orange Drink

Libby's, (pH 2·91 titratable acidity TA 0·80%); orange juice (pH 3·40, TA 0·60%); blackcurrant drink (Ribena RTD), (pH 2·70, TA 0·31%); a baby blackcurrant drink (Baby Ribena), pH 3·53, TA 0·125%); a 0·3% citric acid solution (pH 2·15, TA 0·30%) and distilled water was the negative control. Groups of five enamel specimens were fluoride pre-treated individually into 25 mL of the mouth rinses or 5 g in 20 mL water slurries of the toothpastes before the erosion exposure to citric acid and citric acid – based drinks for one minute.

Human enamel specimens were exposed to acidic solutions for 10, 20 and 30 minutes after pre-treatments with different fluoride products such as Aquafresh (GlaxoSmithKline Consumer Healthcare, Brentford, UK); Macleans sensitive (GalaxoSmithkline Consumer Healthcare, Brentford, UK); Colgate Total (Colgate-Palmolive, Guildford, UK); Plax (Colgate-Palmolive, Guildford, UK); Endekay (Stafford-Miller Ltd, Welwyn Garden City, UK); FluoriGuard (Colgate-Palmolive, Guildford, UK) and Acidulated gel (Dental Products, Loughborough, UK).

The acidic gel was placed directly on enamel specimens for 4 minutes and later on they were subjected to acidic challenge with 0.3% citric acid for 10, 20 and 30 minutes. Finally, the enamel specimens were washed with distilled water and profilometric readings of the test enamel specimens were calculated after 10, 20 and 30 minutes for all the groups except for the acidulated gel group where there were no pre-treatments with the gel for cycles 2 and 3.

Furthermore, the erosive challenge by the acidic soft drinks pre-treated with a fluoride toothpaste, mouthwash or gel showed different levels of enamel surface erosion but the greatest erosion protection was afforded by

pretreatment with acidulated fluoride gel. In comparison to the present study, the experimental design was closer to the daily life scenario regarding the exposure of the enamel specimens to 0.3% citric acid solution for two minutes/ five times per day. That simulated the consumption of 5 snacks either as fruits or fruit juices as recommended (Subar et al., 1995).

All acid solutions either by 0.3% citric acid or the tested commercial acidic drinks demonstrated increased erosion depths with exposure time which is in agreement with the present study that presented enamel surface loss with extended exposure to 0.3% citric acid erosion and tooth brushing abrasion for long period at interval of 7, 14, 21 and 28 days.

The addition of 1ppm F to citric acid or citric acid-based drinks was found to have a reduction in enamel erosion and the authors stated that presumably the effect might be due to an alteration in ion exchange at the surface and did not cause any modification on pH or titratable acidity of the acidic solutions (Hughes et al., 2004). However in the present study, there was no addition of fluoride or metallic ions such as iron into the erosive solution.

4.7 Conclusions

1. Bovine enamel can be used as a substitute for human enamel and showed similar findings in the present study.
2. This *in vitro* procedure successfully demonstrated the use of enamel slabs to determine surface loss under acidic erosion challenge in 0.3% citric acid and tooth brushing abrasion.

3. Different levels of enamel surface loss were observed with different fluoridated toothpastes treatments with the repeated daily exposure to erosive solution under erosive/abrasive procedures despite the presence of similar concentrations of fluoride toothpastes.

4. Toothpastes designed to treat or strengthen gingivitis might not be suitable for individuals who are at risk of erosion.

5. Elmex® Sensitive Plus (AmF, 1400 ppm F), Elmex® *anticaries* (AmF, 1400 ppm F) had significant protection. Amine / stannous AmF/SnF_2 combination had least protective effect, whereas amine (AmF) and sodium fluoride (NaF) toothpastes showed significant protection.

6. Non-fluoridated and anti-gingivitis AmF/SnF_2 toothpastes had the least protective effect against enamel surface loss.

7. This *in vitro* model proved to be an effective standardised laboratory procedure to test specialised products containing different fluoride delivery systems but having similar fluoride concentrations and using the de/ remineralisation cycling process simulating normal daily life (eating and snacking habits) with daily oral hygiene practice.

8. This study proved similarity between human and bovine enamel teeth in erosion / abrasion experimental procedures.

9. Despite the similarity in fluoride concentration, some specialised toothpastes(with special formula) showed enamel surface loss with different effects after extended periods (28 days) of acidic challenge using 0.3% citric acid for 2min/5 times daily and tooth brushing abrasion during toothpaste application 2 min/2 times daily .

10. A significant protection against erosion and abrasion wear were achieved in both bovine and human enamel specimens that were treated with

Elmex® *anti-caries* (E2, AmF, 0.14 F), Elmex® Sensitive (E4, AmF, 0.14 F) and Pronamel (E3, NaF 1450 ppm F) compared to 0 ppm F toothpaste and Meridol® toothpaste containing (AmF/SnF2), 1400 ppm F.

11. The present study reinforces the importance of product formulation in preventing dental surface loss in erosive and abrasive challenges rather than the concentration.

4.8 Recommendations

1. Further research using combined oral products.

2. It is the responsibility of dental health care professionals to advise individuals who are at risk of erosive dental wear to the proper and careful selection of appropriate health care products according to their personal history, medical and clinical dental examination status.

3. Some medicated toothpastes for strengthening gingiva or as anti-plaque or anti-gingivitis might not suitable for individuals who are at risk of dental erosion.

4. Individuals who are more prone to erosion should use mild and properly laboratory tested products and avoid taking over the counter products that may complicate their oral health status.

5 Study 2: The effect of therapeutic products in combination on prevention of tooth surface loss *in vitro*

5.1 Aim

To study the effect of specialised fluoride toothpastes, mouthrinses and other remineralising agents specifically marketed for protection against sensitivity and / or erosion in combination on the surface loss of bovine enamel subjected to citric acid erosion and tooth brushing abrasion *in vitro*.

5.2 Objectives

1. To investigate the effectiveness of twice daily use of two types of anti-erosive combinations (Elmex® Sensitive Plus (AmF) toothpaste plus Elmex® erosion mouthrinse) and Sensodyne® Pronamel® toothpaste and Sensodyne® Pronamel® mouthwash against 0.3% (pH 3.6) citric acidic enamel erosion and tooth brushing abrasion compared to fluoride-free toothpaste (control).
2. To investigate the effectiveness of using two combinations of topical applications (Elmex® sensitive plus (AmF) toothpaste plus Elmex® erosion mouthrinse) and Sensodyne Pronamel® toothpaste and Sensodyne Pronamel® mouthwash on enamel surface loss subjected to both acidic erosion and tooth brushingl abrasion compared to a single application of remineralising agent (GCTooth Mousse).
3. To investigate the effectiveness of using a remineralising agent (GC Tooth Mousse) on treatment of enamel surface loss subjected to both acidic erosion and tooth brushing abrasion versus Fluoride free toothpaste.

5.3 Null hypotheses

1. There are no differences in the effectiveness of twice daily use of two types of anti-erosive combinations (Elmex® Sensitive Plus (AmF) toothpaste plus Elmex® erosion mouthrinse) and Sensodyne Pronamel® toothpaste and mouthrinse combinations against 0.3% citric acidic enamel erosion and tooth brushing procedures compared to fluoride-free toothpaste (control).
2. There are no differences in the effectiveness of using two combinations of topical applications of anti-erosive toothpastes and mouthrinse products on enamel surface loss subjected to both 0.3% citric acidic erosion and tooth brushing abrasion compared to a single application of remineralising agent (GC Tooth Mousse).
3. There are no differences in the effectiveness of using a remineralising agent (GC Tooth Mousse) on treatment of enamel surface loss subjected to both acidic erosion and tooth brushing abrasion compared to fluoride-free toothpaste.

5.4 Materials and methods

5.4.1 Ethical approval as described in section 3.3.1.

5.4.2 Dental hard tissue source and handling as described in section 3.3.2.

5.4.3 Enamel slab preparations and storage as describe in sections 3.3.3.4 and 3.3.3.2 respectively.

5.4.4 Erosive agent and artificial saliva preparations as described in sections 3.3.3.6 and3.3.3.7 respectively.

5.4.5 Experimental design.

The experimental design was performed as the followings;

Table 5.1 Delivery system and specific properties of the oral therapeutic products.

Trade name	Concentration and source of fluoride	Specific properties	Target group	Other key components
Elmex® Sensitive Plus Toothpaste (Elmex TP)	Amine fluoride (AmF) TP; 1400 ppm F	Anti-sensitivity	Dental sensitivity	Hydrated silica, Silica Dimethyl Silylate Hydroxymethylcellulose, polyethelene
Elmex®Erosion protection mouthrinse Elmex MR)	Olafluor, sodium fluoride,(NaF, 500 ppm F)	Erosion protection	People at risk of dental erosion	Stannous chloride $Sncl_2$ (800 ppm Sn_2) Sodium gluconate
Sensodyne® Pronamel® Toothpaste (Pronamel TP)	NaF; 1450 ppm F	Against erosion and helps re-harden enamel.	People at risk of dental erosion	Low abrasivity Neutral pH formula with 5% potassium nitrate (KNO_3)
Sensodyne® Pronamel® daily mouth wash (Pronamel MR)	NaF; 450 ppm F	Helps protect against acid erosion	People at risk of dental erosion	Disodium phosphate Basicand sodium phosphate Contains potassium nitrate
GC Tooth Mousse ™ (GCTM)	0 ppm F	remineralising agent	anti-caries	CPP-ACP, CMC Na
Non-fluoride® (Boots) Toothpaste (control)	0 ppm F	Help maintain healthy gums and freshen the mouth (fresh mint)	Suitable for people who are sensitive to fluoride	Contains sodium bicarbonate

5.4.6 Sample size calculation

Statistical advice was sought and the sample size was calculated by using IBM SPSS of University of Leeds computer system. Assuming use of repeated measures analysis of variance for analysing the data with the standard deviation in control (3.03) from the previous *in vitro* study, the minimum difference to detect 2 (effect size 0.66), intra-cluster correlation (0.981) and power of calculation will be 95% with alpha = 0.05 so the size of 15 samples per group was planned to take into consideration the possible damage or loss in numbers of the slab during the experiments. The outcome is measuring the amount of enamel surface loss in repeated measurements at 7, 14, 21, and 28 days. If the effect size decreased, the sample size would increase and assuming the use of t-test to allow comparison within the groups, at a significance level of 0.05, power 95%, it was determined that the study required 15 samples.

5.4.7 Treatment groupings

Sixty (60) enamel specimens were standardised for hardness, randomly selected, and were mounted in acrylic blocks. They were divided into four experimental groups of 15 samples each per group (number (n)=15 per group) (Table 5.2):

1. Group 1: Elmex® Sensitive Plus toothpaste (Elmex TP)1400 ppm F as AmF followed by Elmex® erosion mouthrinse (MR) as a combination of 500 ppm F (AmF/NaF) and stannous chloride $SnCl_2$ (800 ppm Sn_2) containing rinse 10 ml x 2 times/day (Elmex TP plus MR).
2. Group 2: Sensodyne® Pronamel toothpaste 1,450 ppm F as NaF and Sensodyne® Pronamel mouthwash contained NaF 450 ppm F mouthrinse 10 ml x 2 times/day (Pronamel toothpaste (TP) plus mouthrinse (MR).

3. Group 3: Sensodyne® Pronamel toothpaste1,450 ppm F as NaF + GC Tooth Mousse™ (TM) once per day (Pronamel TP plus TM).

4. Group 4: non-fluoride® (Boots) 0 ppm F as a control.

The erosive challenges were achieved using citric acid (0.3%, pH 3.6) for 2 min 5 times/day followed by 1 h in artificial day time saliva (pH 6.8) between the erosive challenges. Slabs were subjected to 2 min brushing abrasion twice per day with 1: 3 toothpaste / day artificial saliva slurry using a 300 g load. At all other times the samples were incubated in night time artificial saliva. The enamel slabs were rinsed thoroughly with tap water after each experimental procedure. The assessment of the amount of enamel surface loss (µm) was made using profilometry (Proscan 2000, UK) at periodic intervals after 7, 14, 21, and 28 days.

The distribution of the test groups and related treatment procedures are shown in Table 5.2.

Table 5.2 Treatment groupings and erosive - abrasive cycling procedures

Group	Treatment
Group1: Elmex®Sensitive Plus toothpaste (AmF, 0.14%) plus Elmex® Erosion mouthrinse (Elmex TP plus MR)	Slurry from Elmex®Sensitive Plus toothpaste (AmF,0.14% F) / day time artificial saliva formulation was applied for 2 mins and 15 strokes using a 300 g load tooth brushing procedure. Followed by application Elmex® Erosion mouthrinse containing F and stannous chloride rinse 10 ml x 2 times/day). Using in vitro horizontal manual shaking inside the plastic container for 30 seconds (the rinsing time as per manufacturers' instructions).
Group2: Sensodyne® Pronamel® Toothpaste plus Sensodyne® Pronamel® mouthrinse (Pronamel TP plus MR)	Slurry from Sensodyne® Pronamel toothpaste (1,450 ppm F, NaF) / day time artificial saliva formulation was applied for 2 mins and 15 strokes using a 300 g load tooth brushing procedure. Followed by application of Sensodyne® Pronamel® (NaF 450 ppm F mouthrinse) 10 ml x 2 times/day. Using a horizontal manual shaking inside the plastic container for 1 minute (the rinsing time as per manufacturers' instructions).
Group3: Sensodyne® Pronamel® Toothpaste,1450 ppm F as NaF plus GC Tooth Mousse™	Slurry from Sensodyne® Pronamel toothpaste, NaF (0.14% F) / day time artificial saliva formulation was applied for 2 mins with 15 strokes using a 300 g load tooth brushing procedure, and then a single application of GC Tooth Mousse once a day for 5 minutes at the end of each experimental day for consecutive 7 days.
Group4: non-fluoride® (Boots) (0ppm F) as a control	Slurry from non- fluoride® toothpaste / day time artificial saliva formulation was applied for 2 mins and 15 strokes using a 300 g load tooth brushing procedure.

5.5 Results

5.5.1 Distribution of the data

The normality of distribution of the data was checked using the Kolmogrov-Smirov test. All data were normally distributed when 2-tailed $p>0.05$ for all groups and at the end of each experimental procedure after 7, 14, 21 and 28 days as shown in Table 5.3.

Table 5.3 Distribution of data analysis of bovine enamel surface changes using non-parametric Kolmogorov-Smirnov test.

One-Sample Kolmogorov-Smirnov Test

ESL (µm) after 7 days		
N		60
Normal Parameters[a,b]	Mean	.7158
	Std. Deviation	.649
Most Extreme Differences	Absolute	.152
	Positive	.152
	Negative	-.135
Kolmogorov-Smirnov Z		1.175
Asymp. Sig. (2-tailed)		.126

a. Test distribution is Normal.
b. Calculated from data.

One-Sample Kolmogorov-Smirnov Test

ESL (µm) after 14 days		
N		60
Normal Parameters[a,b]	Mean	.993
	Std. Deviation	1.09
Most Extreme Differences	Absolute	.214
	Positive	.214
	Negative	-.187
Kolmogorov-Smirnov Z		1.66
Asymp. Sig. (2-tailed)		.091

a. Test distribution is Normal.
b. Calculated from data.

One-Sample Kolmogorov-Smirnov Test

ESL (µm) after 21days		
N		60
Normal Parameters[a,b]	Mean	1.08
	Std. Deviation	1.169
Most Extreme Differences	Absolute	.219
	Positive	.219
	Negative	-.190
Kolmogorov-Smirnov Z		1.696
Asymp. Sig. (2-tailed)		.076

a. Test distribution is Normal.
b. Calculated from data.

One-Sample Kolmogorov-Smirnov Test

ESL (µm) after 28 days		
N		60
Normal Parameters[a,b]	Mean	1.37
	Std. Deviation	1.55
Most Extreme Differences	Absolute	.235
	Positive	.235
	Negative	-.206
Kolmogorov-Smirnov Z		1.823
Asymp. Sig. (2-tailed)		.063

a. Test distribution is Normal.
b. Calculated from data.

5.5.2 Distributions of enamel surface loss (µm) changes from baseline

Distribution of the experimental groups during the study period of 0.3% (pH 3.6) citric acidic erosion and twice daily tooth brushing abrasion with the corresponding combination of therapeutic test products are presented in (Table 5.4).

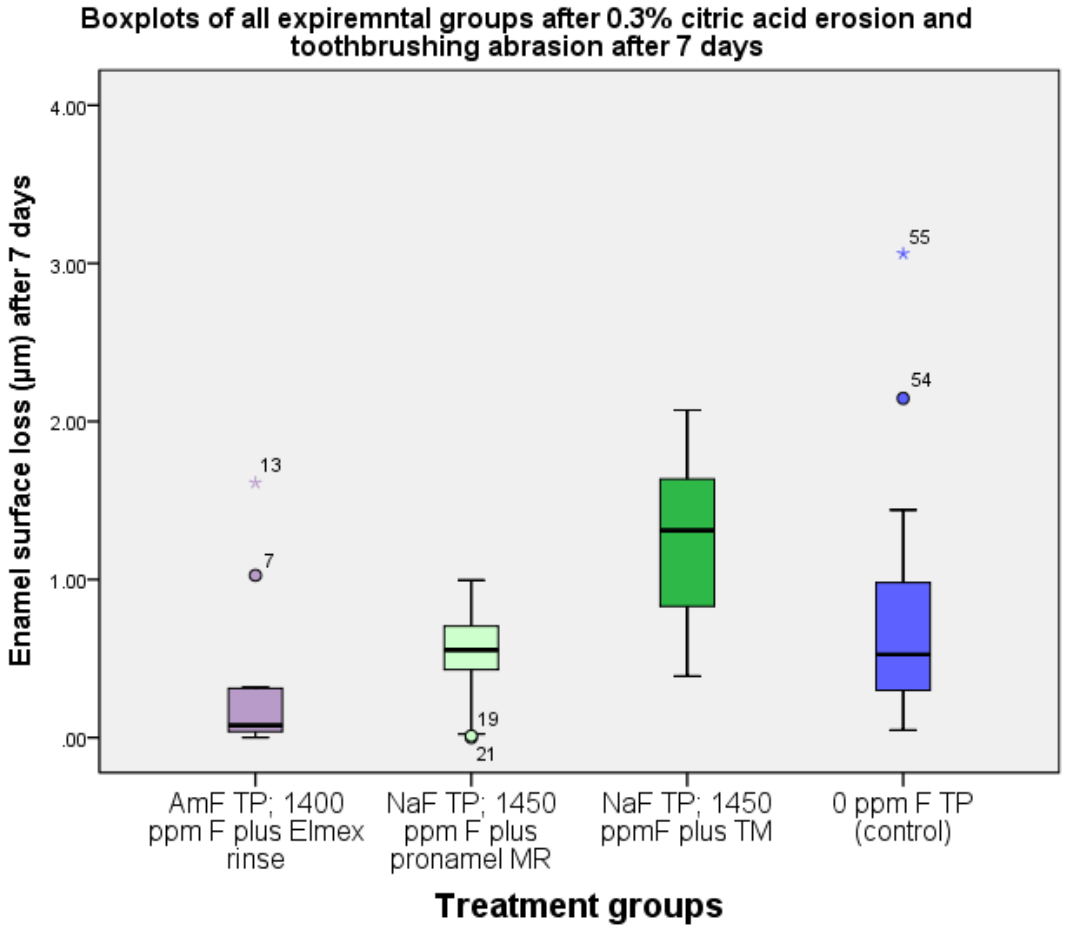
The results after 7 days present insignificant minimal changes among the therapeutic groups compared to the control group. After 14 days, the lowest minimal enamel surface loss (µm) is observed in the therapeutic combination with Elmex toothpaste plus Elmex erosion mouthrinse (mean ± standard deviation) is 0.34 ± 0.28 µm, then for Sensodyne Pronamel toothpaste and Pronamel mouthwash against erosion 0.61 ± 0.49 µm compared to Pronamel toothpaste and Tooth Mousse and control groups. After 21 days the lowest minimal enamel mean surface loss continued to be observed with similar unchanged values 0.34 ± 0.29 µm for the Elmex group and 0.57 ± 0.49 µm for the combined Pronamel toothpaste and Pronamel mouthwash group compared to either Pronamel, Tooth Mousse or the control groups.

At the end of de/remineralisation and repeated treatments, the results of mean changes for the erosive and abrasive enamel surface loss demonstrated the lowest minimal enamel surface loss in the Elmex group (0.40 ± 0.23 µm) and Pronamel toothpaste combined with Pronamel mouth wash (0.60 ± 0.28 µm) compared to the other groups.

Table 5.4 Distribution of erosive surface loss (µm) changes caused by the different test therapeutic products during experimental periods at 7, 14, 21 and 28 days.

	Test products	Mean ± St. deviation	Median	Minimum	Maximum	Confidence interval 95%	
						Lower bound	Upper bound
After 7 days	Elmex®Sensitive toothpaste plus Elmex® Erosion mouthrinse	0.29 ± .45	0.1	.00	1.61	0.04	0.54
	Sensodyne® Pronamel® Toothpaste plus Sensodyne® Pronamel® mouthrinse	0.52 ± 0.31	0.55	.00	1.00	0.35	0.69
	Sensodyne® Pronamel® Toothpaste plus GCTooth Mousse	0.27 ± 0.52	1.31	0.39	2.07	0.94	1.51
	non-fluoride® (Boots) (0ppm F as a control)	0.83 ± 0.83	0.53	0.05	3.06	0.38	1.29
After 14 days	Elmex®Sensitive toothpaste plus Elmex® Erosion mouthrinse	0.34 ± .28	0.2	0.02	1.0	0.18	0.50
	Sensodyne® Pronamel® Toothpaste plus Sensodyne® Pronamel® mouthrinse	0.61 ± 0.49	0.6	0.02	1.80	0.34	0.88
	Sensodyne® Pronamel® Toothpaste plus GCTooth Mousse™	1.80 ± 1.10	1.6	0.39	3.91	1.19	2.41
	non-fluoride® (Boots) (0ppm F as a control)	1.20 ± 1.47	0.8	0.16	5.94	0.38	2.01
After 21 days	Elmex®Sensitive toothpaste plus Elmex® Erosion mouthrinse	0.34 ± .29	0.5	0.06	1.02	0.18	0.81
	Sensodyne® Pronamel® Toothpaste plus Sensodyne® Pronamel® mouthrinse	0.57 ± 0.49	0.5	0.02	1.80	0.33	0.88
	Sensodyne® Pronamel® Toothpaste plus GCTooth Mousse	2.29 ± 1.39	1.9	0.61	5.73	1.52	3.05
	non-fluoride® (Boots) (0ppm F as a control)	1.13 ± 1.07	0.9	0.40	4.70	0.38	2.01
After 28 days	Elmex®Sensitive toothpaste plus Elmex® Erosion mouthrinse	.40 ± 0.23	0.5	0.10	0.74	0.28	0.35
	Sensodyne® Pronamel® Toothpaste plus Sensodyne® Pronamel® mouthrinse	.60 ± 0.28	0.50	0.02	1.80	0.33	0.88
	Sensodyne® Pronamel® Toothpaste plus GCTooth Mousse	2.56 ± 1.61	1.56	0.39	2.07	0.30	3.92
	non-fluoride® (Boots) (0ppm F as a control)	1.87 ± 1.84	0.81	0.16	5.94	0.38	2.01

5.5.3 Changes of bovine enamel surface loss (µm) of all experimental groups after 7 days.

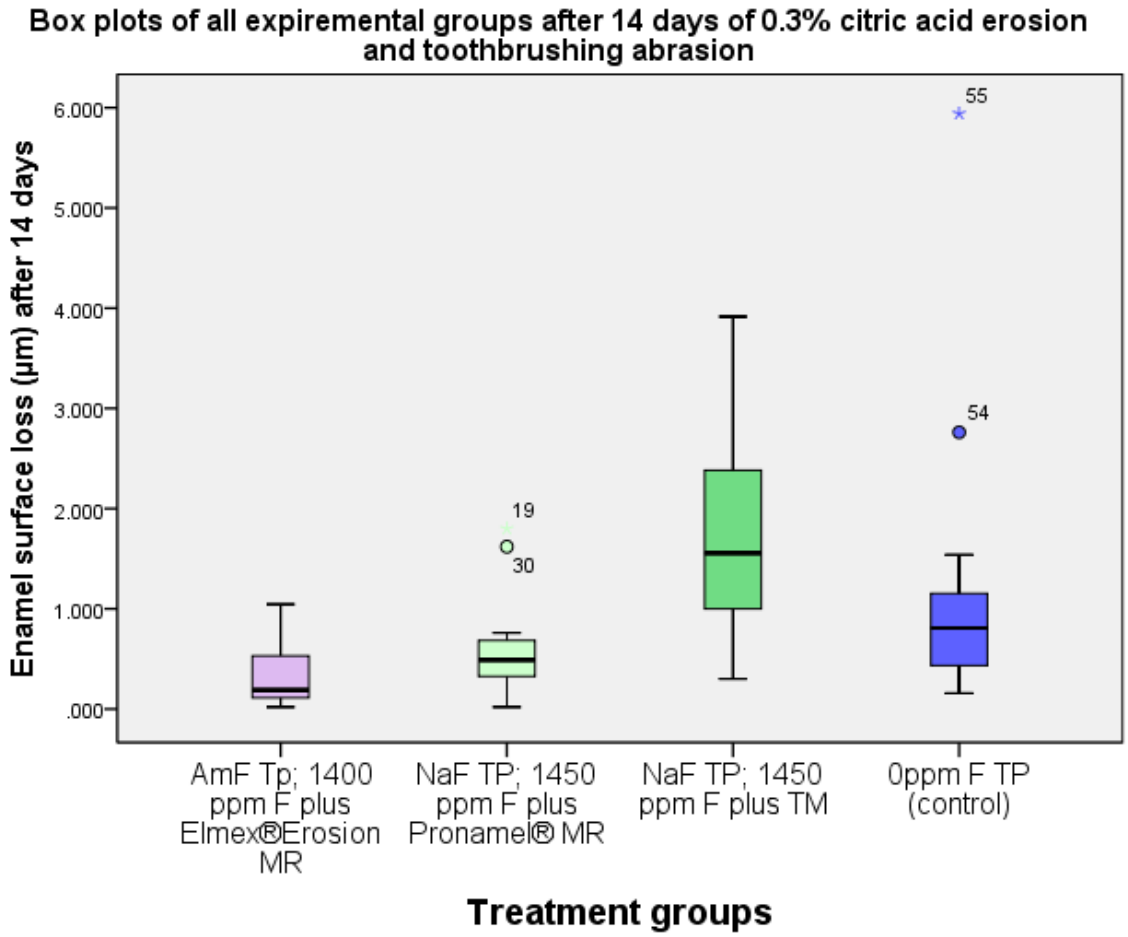


*** and o are outliers and extremes values**

Figure 5.1 Changes of bovine enamel surface loss (µm) caused by four therapeutic test products under 0.3% citric acid erosion and tooth brushing abrasion after 7 dys cycling.

Figure 5.1 shows the minimum median change for the first group of Elmex erosion toothpaste (AmF TP; 1400 ppm F) plus Elmex erosion mouthrinse (Elmex MR) compared to all other experimental groups. Sensodyne® Pronamel toothpaste (NaF TP, 1450 ppm F) with Sensodyne® Pronamel mouthrinse (Pronamel MR) demonstrated a similar median change value trend with 0 ppm F TP and a lower median change value than when Sensodyne® Pronamel toothpaste was combined with GC Tooth Mousse™ (TM).

5.5.4 Changes of enamel surface loss (µm) of all experimental groups after 14 days.

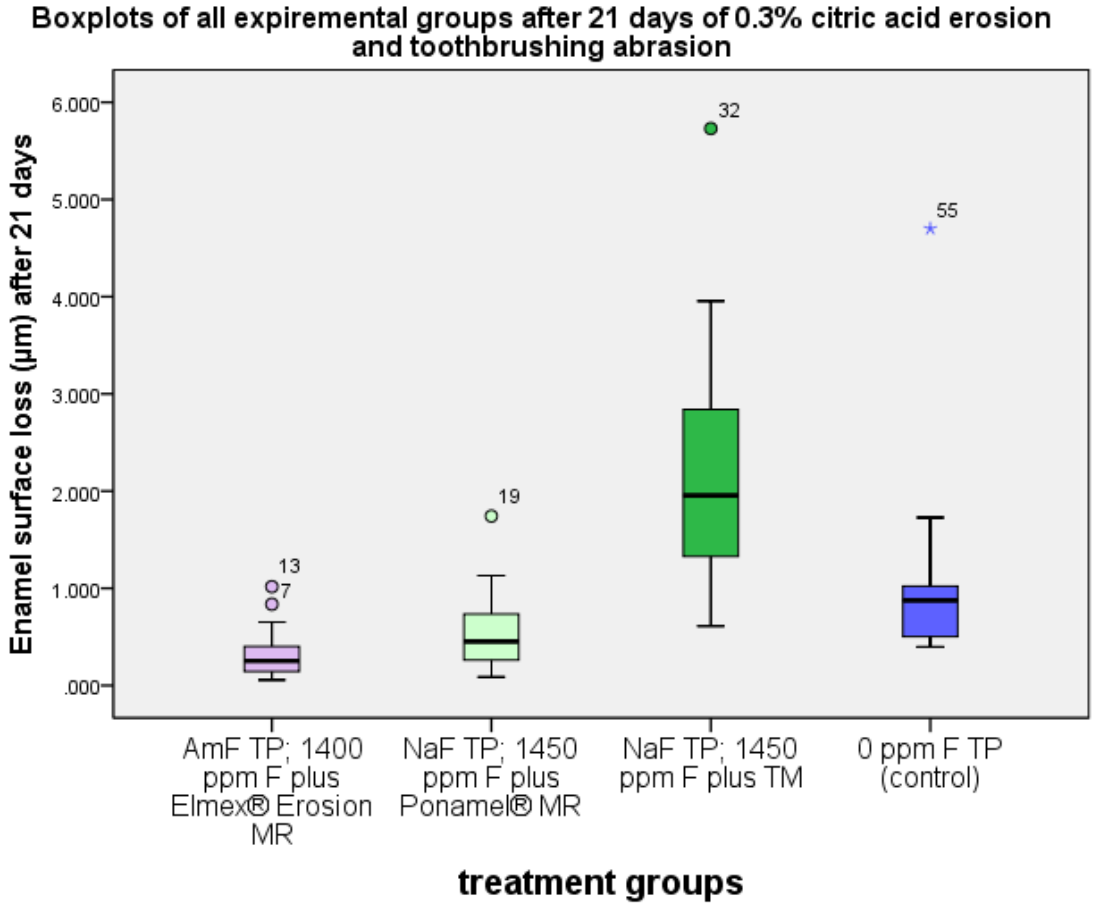


* and o are outliers and extremes values

Figure 5.2 Changes in bovine enamel surface loss (µm) caused by four therapeutic test products under 0.3% citric acid and tooth brushing abrasion after 14 days.

The effect of the therapeutic products on 0.3% citric acid erosion and twice daily tooth brushing abrasion was observed after 14 days of erosive and abrasive cycling procedures is shown in Figure 5.2 which shows the lowest median change for the Elmex TP, 1400 ppm F plus Elmex erosion mouth rinse group, secondly for Pronamel toothpaste, 1450 ppm F plus Pronamel (MR) group compared to the other groups of combined Pronamel toothpaste, GCTooth Mousse (TM) and the control.

5.5.5 Changes of enamel surface loss (µm) of all experimental groups after 21 days.

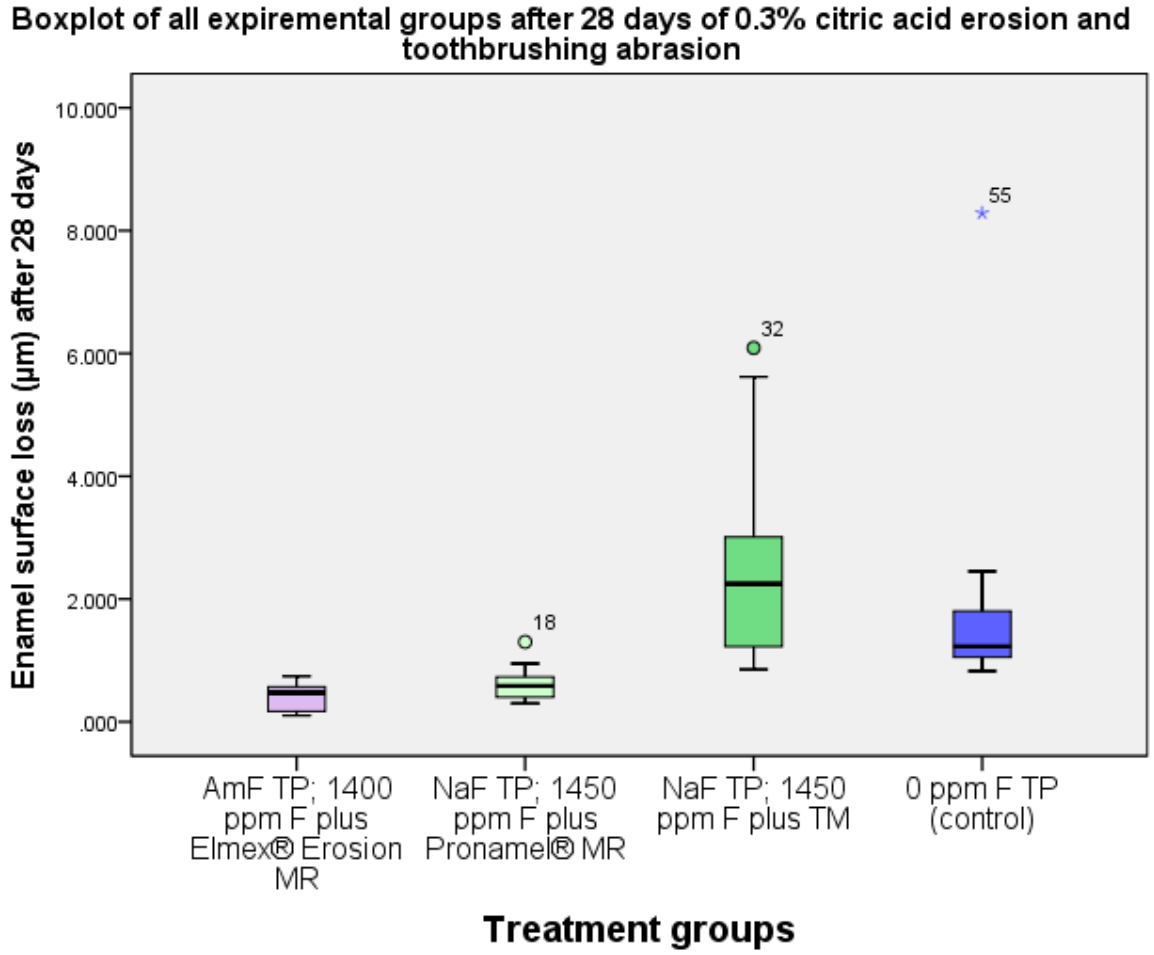


* and o are outliers and extremes values.

Figure 5.3 Changes in bovine enamel surface loss (µm) caused by four therapeutic test products under 0.3% citric acid and tooth brushing abrasion after 21 days.

The effect of the therapeutic products on 0.3% citric acid erosion and twice daily tooth brushing abrasion was observed after 21 days of erosive and abrasive cycling procedures. Figure 5.3 shows the lowest median change for the Elmex TP, 1400 ppm F plus Elmex erosion mouth rinse group, and secondly for the Pronamel toothpaste, 1450 ppm F plus Pronamel (MR) group compared to the other groups of combined Pronamel toothpaste and GCTooth Mousse (TM) and the control.

5.5.6 Changes of enamel surface loss (µm) of all experimental groups after 28 days.



*** and o are outliers and extremes values.**

Figure 5.4 Changes in bovine enamel surface loss (µm) caused by four therapeutic test products under 0.3% citric acid and tooth brushing abrasion after 28 days.

The effect of the therapeutic products on 0.3% citric acid erosion and twice daily tooth brushing abrasion was observed after 28 days of erosive and abrasive cycling procedures. Figure 5.4 shows the lowest median change for the Elmex TP, 1400 ppm F plus Elmex erosion mouth rinse group, and for the Pronamel toothpaste, 1450 ppm F plus Pronamel (MR) group compared to the other groups of combined Pronamel toothpaste and GCTooth Mousse (TM) and the control.

5.5.7 ANOVA significance test results between the experimental groups

For statistical handling; ESL (µm) 1 refers to enamel loss after 7 days, ESL (µm) 2 after 14 days, ESL (µm) 3 after 21 days and ESL (µm) 4 after 28 days.

Table 5.5 displays the analysis of variance (ANOVA) significance tests between the treatment groups at various experimental time periods of 7 days, 14 days, 21 days and at the end of 28 days which shows highly significant treatment effects between the groups at all different experimental periods ($p \leq 0.000$). The significance level was set up initially at 0.05 using (post hoc, LSD at 0.05), and then for Bonferroni test the significance level was calculated for a p value set at 0.005.

Therefore as the significance level results showed $p<0.5$, the null hypothesis was rejected (Table 5.5).

There were significant differences between the treatment groups and changes in ESL (µm) for all experimental periods at 7, 14,21 and 28 days.

Table 5.5 ANOVA test to evaluate the significant differences between and within the groups.

		Sum of Squares	Df	Mean Square	F	P value
ESL (µm) after 7 days	Between Groups	7.45	3	2.48	7.99	.000
	Within Groups	17.38	56	.31		
ESL (µm) after 14 days	Between Groups	18.50	3	6.16	6.64	.001
	Within Groups	51.94	56	.93		
ESL (µm) after 21 days	Between Groups	33.98	3	11.33	13.60	.000
	Within Groups	46.63	56	.83		
ESL (µm) after 28 days	Between Groups	49.87	3	16.62	10.22	.000
	Within Groups	91.12	56	1.63		

Table 5.6 presents the statistical analysis with LSD (post hoc) that showed a higher significance for combinations of Elmex toothpaste plus Elmex mouthrinse and therapeutic combinations of Pronamel TP plus Pronamel mouthrinse compared with Pronamel toothpaste plus TM $p \leq 0.001$ and $p < 0.001$ respectively, and with the control $p < 0.01$, whereas, no significance was observed in group 3 (Pronamel TP and Pronamel MR), or group 4 $p \leq 0.1$.

Table 5.6 Comparisons between the effects of four therapeutic test products on enamel surface loss caused by 0.3% citric acid erosion and tooth brushing abrasion after 7 days.

Comparisons between the treatment groups		Mean Difference	Std. Error	p value	95% Confidence Interval	
					Lower Bound	Upper Bound
Group 1 AmF TP; 1400 ppm F plus (Elmex ®Erosion) MR	NaF TP plus Pronamel® MR	-.23	.20	.266	-.64	.18
	NaF TP plus GC TM	-.94*	.20	.000	-1.35	-.53
	0 ppm F TP (control)	-.55*	.20	.010	-.95	-.14
Group 2 NaF TP; 1450 ppm F plus Pronamel® MR	NaF TP; 1450 ppm F plus GC TM	-.71*	.20	.001	-1.12	-.30
	0 ppm F TP (control)	-.32	.20	.126	-.72	.09
Group 3 NaF TP; 1450 ppm F plus GC TM	0 ppm F TP (control)	.39	.20	.058	-.015	.801
Group 4 0 ppm F TP (control)	AmF TP; 1400 ppm F plus (Elmex ®) MR	.55*	.20	.010	.14	.95
	NaF TP; 1450 ppm F plus Pronamel® MR	.32	.20	.126	-.09	.72

* Mean value is significant at the 0.05 level.

In Table 5.7 the statistical analysis with LSD (post hoc) showed a higher significance for combinations of Elmex toothpaste plus Elmex mouthrinse and therapeutic combinations of Pronamel TP plus Pronamel mouthrinse compared with Pronamel toothpaste plus TM $p \leq 0.001$ and with the control $p < 0.02$, whereas, no significance was observed between Pronamel TP and Pronamel MR, or the control group $p = 0.10$. No statistical significance was observed between the control and Pronamel TP and TM $p > 0.05$.

Table 5.7 Comparisons between the effects of four therapeutic test products on enamel surface loss (µm) caused by 0.3% citric acid and tooth brushing abrasion after 14 days.

Comparisons between the treatment groups		Mean Difference	Std. Error	p value	95% Confidence Interval	
					Lower Bound	Upper Bound
AmF TP; 1400 ppm F plus (Elmex ® Erosion) mouthrinse (MR)	NaF TP; 1450 ppm F plus GC TM	-1.44*	.35	.000	-2.14	-.73
	0 ppm F TP (control)	-.83*	.35	.022	-1.54	-.126
NaF TP; 1450 ppm F plus Pronamel® MR	AmF TP; 1400 ppm F plus (Elmex ®Erosion) MR	.25	.35	.486	-.46	.95
	NaF TP; 1450 ppm F plus GC TM	-1.19*	.35	.001	-1.89	-.48
NaF TP; 1450 ppm F plus GC ™ (TM)	0 ppm F TP (control)	.61	.35	.091	-.099	1.31
0 ppm F TP (control)	AmF TP; 1400 ppm F plus (Elmex ®Erosion) MR	.83*	.35	.022	.126	1.54
	NaF TP; 1450 ppm F plus Pronamel® MR	.58	.35	.103	-.121	1.29

* Mean value is significant at the 0.05 level.

In Table 5.8 the statistical analysis with LSD (post hoc) shows a higher significance for combinations of Elmex toothpaste plus Elmex mouthrinse and therapeutic combinations of Pronamel TP plus Pronamel mouthrinse compared with Pronamel toothpaste plus TM ($p \leq 0.001$). However, for the control the significance was ($p < 0.02$), whereas there wasn't any significance between Pronamel TP and Pronamel MR or the control group ($p > 0.05$). Statistical significance was observed between the control and Pronamel TP and TM ($p \leq 0.01$).

Table 5.8 Comparisons between 4the effects of four therapeutic test products on enamel surface loss caused by 0.3% citric acid and tooth brushing abrasion after 21 days.

Comparisons between the treatment groups		Mean Difference	Std. Error	p value	95% Confidence Interval	
					Lower Bound	Upper Bound
AmF TP; 1400 ppm F plus (Elmex®Erosion) MR	NaF TP; 1450 ppm F plus Pronamel® MR	-.23	.33	.487	-.901	.434
	NaF TP; 1450 ppm F plus TM	-1.95*	.33	.000	-2.62	-1.28
	0 ppm F TP (control)	-.79*	.33	.022	-1.46	-.121
NaF TP; 1450 ppm F plus Pronamel® MR	NaF TP; 1450 ppm F plus TM	-1.72*	.33	.000	-2.38	-1.05
	0 ppm F TP (control)	-.56	.33	.101	-1.22	.113
NaF TP; 1450 ppm F plus GC TM	AmF TP; 1400 ppm F plus (Elmex®) MR	1.95*	.33	.000	1.28	2.62
0 ppm F TP (control)	AmF TP; 1400 ppm F plus (Elmex®) MR	.79*	.33	.022	.12	1.46
	NaF TP; 1450 ppm F plus Pronamel® MR	.56	.33	.101	-.113	1.22
	NaF TP; 1450 ppm F plus TM	-1.16*	.33	.001	-1.83	-.49

* Mean value significant at the 0.05 level.

In Table 5.9 the statistical analysis with LSD (post hoc) shows a higher significance for both groups of combinations of Elmex toothpaste plus Elmex mouthrinse and therapeutic combinations of Pronamel TP plus Pronamel mouthrinse compared with Pronamel toothpaste plus TM ($p \leq 0.001$). However, the Elmex group combinations showed a greater significance compared with the control ($p < 0.002$), and between Pronamel TP and Pronamel MR and the control group ($p \leq 0.01$). There was no statistical significance between the control and Pronamel TP and TM ($p > 0.05$).

Table 5.9 comparisons between the effects of four therapeutic test products on enamel surface loss (µm) caused by 0.3% citric acid and tooth brushing abrasion after 28 days.

Comparisons between the treatment groups		Mean Difference	Std. Error	p value	95% Confidence Interval	
					Lower Bound	Upper Bound
AmF TP; 1400 ppm F plus (Elmex®Erosion) MR	NaF TP; 1450 ppm F plus TM	-2.21*	.47	.000	-3.14	-1.28
	0 ppm F TP (control)	-1.47*	.47	.003	-2.40	-.535
NaF TP; 1450 ppm F plus Pronamel® MR	AmF TP; 1400 ppm F plus (Elmex®Erosion) MR	.196	.47	.675	-.737	1.13
	NaF TP; 1450 ppm F plus TM	-2.01*	.47	.000	-2.95	-1.08
	0 ppm F TP (control)	-1.27*	.47	.008	-2.21	-.34
NaF TP; 1450 ppm F plus GC TM	NaF TP; 1450 ppm F plus Pronamel® MR	2.01*	.47	.000	1.08	2.95
	0 ppm F TP (control)	.742	.47	.117	-.19	1.68
0 ppm F TP (control)	AmF TP; 1400 ppm F plus (Elmex®Erosion) MR	1.47*	.47	.003	.54	2.40
	NaF TP; 1450 ppm F plus Pronamel® MR	1.27*	.47	.008	.34	2.21

* Mean value significant at the 0.05 level.

5.6 Discussion

5.6.1 The rationale for this study

The literature review revealed different study protocols exist for investigating erosive / abrasive procedures. It was shown that different erosive challenges to demineralise dental tissue lesions such as exposing the dental samples in the demineralising agent for an unrealistically long time period (e.g. erosion of enamel in some soft drinks and orange juice for 7 days or 24 hours or in another study exposing groups of specimens to 0.3% citric acid (pH 3.2) for 30 min, 1, 2, 3 or 4 hours (Larsen and Nyvad, 1999, Eisenburger et al., 2000) or (2) to expose the samples alternating in an acidic soft drink as a demineralising solution (for 5 min) and a remineralising solution of artificial saliva (for 1min) four times daily, then the specimens were brushed in an automatic brushing machine (2,000 strokes, 2.5 N load) and subsequently stored again in saliva (1 min). In that study to evaluate the abrasion resistance of the eroded enamel using highly concentrated gels for 30 seconds after brushing, the slurry was removed from the specimens by rinsing with distilled water. For each of 16 specimens the following gels (A-D) were used: gel A (pH 7.0) and gel B (pH 4.5) were non-fluoridated; gels C (pH 7.0) and D (Elmex gelee; pH 4.5) contained 1.25% F-. After two cycles the specimens were kept in the saliva for 8 h. Finally the tape was removed and the abrasion was determined. However the results of that study showed that the treatment of enamel erosions with an acidified fluoride gel resulted in abrasion resistance against erosion compared to the non-fluoridated or neutral gels (Attin et al., 1999).

Contrary to this some erosive and abrasive *in vitro* and *in situ* studies that evaluated dental wear resistance showed there was insignificant reduction of tooth wear after applying highly concentrated dentifrices with 5000 ppm F (Rios et al., 2008b, Magalhaes et al., 2008c). Also applying highly concentrated fluoride in the form of sodium fluoride varnish as Duraphat-D (NaF, 2.26%F), Duofluorid-F (NaF, 2.71% F) or TiF(4)-T (2.45%F) did not reduce the enamel wear (Magalhaes et al., 2007b, Moretto et al., 2010). However, there is no doubt that fluoride used in high concentrations and amounts is toxic and can be fatal although dentally reported fatalities are extremely rare (Newbrun, 1987, Whitford, 1992). Furthermore, these oral care products are not convenient for self-administered daily home practices and applying highly concentrated fluoride varnish or gels requires professional applications in dental clinical practice. Moreover using a fluoride mouthrinse gives more fluoride retention in the oral environment (Zero et al., 1988b). Based on the comparison between the use of dentifrices or mouthrinses in the retention of fluoride in the oral environment over 24 hours was demonstrated in 10 adults between 18- 52 years who brushed and/or rinsed twice per day in the morning and before bed with either a placebo dentifrice (8 ppm F), NaF dentifrice (1100 ppm F), or NaF rinse (225 ppm F). Experimental procedures were performed with placebo dentifrice only (PD); F dentifrice only (FD); F dentifrice followed by F rinse (FD/FR); placebo dentifrice followed by F rinse (PD/FR); and F rinse followed by placebo dentifrice (FR/PD). Unstimulated whole saliva samples were collected at baseline and at 0, 15, 30, and 45 min, 1, 2, and 8 hr after brushing with rinse in the morning and after B/R in the evening, then upon rising the following morning. Salivary flow and fluoride rates were measured for each sampling interval. The results of the study showed that NaF (225 ppm F) rinse was a more effective way of delivering topical fluoride than

fluoride toothpaste or both placebo dentifrice and rinse but fluoride rinse only led to longer retention in the oral environment for the older subjects during bedtime application (Zero et al., 1988b).

In addition, using a combination of an amine fluoride/sodium fluoride mouthrinse (total F = 250 ppm) in addition to an amine fluoride dentifrice (F = 1,250 ppm) provided a significant increase in both KOH–soluble and structurally bound fluoride in enamel and dentine when a fluoride mouthrinse was used (van Strijp et al., 1999).

Fluoride mouth rinses for use at home or in school-based programs are currently popular as a simple and safe way to expose teeth to fluoride at concentrations of about 0.02%. The recommended daily application of 10 mL volume for all of these rinses contains less than 2.5 mg of fluoride, a safe amount, even if they are accidentally swallowed.

It was proposed that to reach the safely tolerated dose (STD) limit, the hypothetical 2-year-old child would have to swallow at one time 360 mL (12 oz) of a neutral NaF rinse and, to reach the certainly lethal dose (CLD) about 5 to 10 g of sodium fluoride needs to be ingested. However, one quarter of the certainly lethal dose can be ingested without causing serious acute toxicity and is known as the safely tolerated dose (STD) about 1.5 quart of rinse. The largest commercially available bottle of a NaF rinse contains about 509 ml or 118 mg of fluoride, an amount greater than the STD of fluoride but well below the CLD for a 2-year-old child. It is unlikely, however, that a child could consume the entire 509 ml without vomiting (Heifetz and Horowitz, 1986).

5.6.2 Discussion of combined toothpaste and mouthrinse groups

Multivariate analyses revealed that fluoride rinsing and tooth brushing lessons at primary school for at least 3 years (besides the educational level of parents) was the most determining factor for tooth status independent of other variables (Pieterse et al., 2006). Subjects who never used fluoride mouthrinse were almost four times more likely to have carious lesions than subjects who rinsed for at least 3 years and it was strongly indicated that long-term rinsing with fluoride had a positive effect on tooth status (Pieterse et al., 2006), mouthrinses (over 50%) once or more daily and oral irrigators (55%) on a regular basis (Bakdash, 1995).

It was demonstrated that 2.02% NaF solution at pH 1.2 was able to reduce calcium release by 38% after 1-min erosion in hydrochloric acid (pH 2.6) and by 17% after 10-min erosion, but not to significantly reduce enamel surface loss as seen using profilometry and cross-sectional SEM images. However, enamel surface loss reduction following the application of 1.50% TiF_4 (titanium tetrafluoride) solution at pH 1.2 was 30% after 1 min and 16% after 10 min of erosion (Wiegand et al., 2009b). The protective effect of NaF was related to the formation of a CaF_2-like surface precipitates, which were shown to be significantly enhanced under acidic conditions as well as with increasing length of fluoride exposure and fluoride concentration (Ganss et al., 2007). This loosely bound fluoride might protect the surface to a certain extent against demineralisation as it acts as a reservoir for fluoride which facilitates the precipitation of minerals by forming fluorapatite or fluorohydroxyapatite, thereby preventing further loss of mineral ions (Rølla et al., 1993). However, even though enamel and dentine analysis revealed higher amounts of fluoride in the samples treated with NaF, especially at pH 1.2, SEM pictures did not show the

deposition of loosely bound fluoride in the form of globular precipitates. Similarly, (Schlueter et al., 2007) found that 2.2% NaF solution at pH 1.2 reduced enamel erosion to approximately 50% in a 5-day de- and remineralisation cycle. However, 1.64% w/v TiF4 was also shown to provide better protection.

The frequent application of NaF led to better protection against enamel erosion (Wiegand et al., 2009b).

The efficacy of mouthrinses containing fluoride preparations of 100 µg F/ ml, 225 µg F / ml and a combination of the addition of fluoride (100 µg/ml) to 0.2% sodium trimetaphosphate (TMP) (as a chelating agent) was evaluated on the reduction of bovine enamel dental erosion for a duration of 5 days which were subjected to erosive challenges (unstirred soft drink, degassed, Sprite Zero, Brazil, pH 2.8), 4 times per day (5 min each, 5 ml/block). Following the first and last erosive challenge of each day the blocks were treated with one of the mouth rinses (5 ml/block, 30 s) at room temperature, followed by a 1-hour remineralising period in unstirred artificial saliva. The blocks were washed with de-ionised water before each step to prevent contamination of the solutions. At the end of each day, the blocks were stored in artificial saliva at 37 C. Subsequent to the erosive challenges and treatments with the prepared formulations of mouth rinses only the formula that contained the addition of 0.2% TMP was able to increase the enamel erosion prevention capacity of F (100 µg /g) by 40% compared to groups containing 100 and 225 fluoride, which did not contain TMP (Manarelli et al., 2011).

In the present study, it was shown using the combined therapeutic products of toothpastes and mouthrinses with specialised advanced formulae such as Elmex ® Sensitive Plus toothpaste in combination with Elmex® erosion mouthrinse, and also

with the combined use of Sensodyne Pronamel toothpaste plus Pronamel mouthrinse to protect and treat the repeated erosive attacks. The results of the present study demonstrated a promising effective therapeutic protection against daily repeated erosive exposure to dental enamel rather than using highly concentrated fluoride products (Rios et al., 2008b, Ren et al., 2009) or intensive fluoridation in which using combinations of fluoride toothpaste plus fluoride mouthrinse and fluoride gel (Ganss et al., 2001b), which consequently, of high concentration intake fluoride may lead to dental and bone fluorosis (Dhar and Bhatnagar, 2009).

In the present study, the experimental toothpastes and mouthrinses were within the conventional range of permitted fluoride concentrations (AmF; 1400 ppm F, and NaF; 1450 ppm F).

The intensive application of fluoridation measures consisted of a combination of toothpaste plus mouthrinse and fluoride gel was suggested, demonstrated and found to be effective in reducing tooth wear over 5 days of de/ remineralisation cycling procedures (Ganss et al., 2001b). However the present study used therapeutic products against erosion over an extended period for 28 days.

The effect of fluoride on the progression of erosive demineralisation by immersion of human enamel and dentine in 0.05 *M* citric acid (pH 2.3) for 6×10 mins/day and then, were stored in a remineralisation solution using a cycling de– and remineralisation model in vitro. The mineral loss was calculated daily by longitudinal microradiography (LMR) and presented as cumulative mineral loss over 5 days. Fluoridation measures were performed in 3 groups**:** group 1: control, no fluoridation; group 2: toothpaste fluoridation 3×5 min/daily NaF, 0.15% F; group 3: toothpaste

fluoridation as group 2 and additionally application of a fluoride mouthrinse Olaflur/SnF, 0.025%F (3×5) min/daily and on days 1 and 3 gel fluoridation Olaflur/NaF; 1.25% F for 1×5 mins. After 5 days the lowest erosive mineral loss values for enamel with intensive fluoridation significantly reduced erosion progression in enamel but had a more pronounced effect on dentine ($p \leq 0.001$). The investigators advised that subjects with erosive lesions should use an intensive fluoridation measure (Ganss et al., 2001b).

The influence of stannous fluoride ion concentrations in various experimental solutions on erosion progression in human enamel specimens in which they were subjected to a cyclic de- and remineralisation procedure for 10 days, with six demineralisation periods per day, of 5 min each was investigated. Erosive demineralisation was performed with 0.05 M citric acid (pH 2.3). Except in the control group, specimens were treated for 2 min with test solutions after the first and the sixth demineralisation. Test solutions containing: 1500 mg/L F^- were divided into 4 groups: group 1: 2800 mg/L $Sn^{2+;}$ group 2: 2100 mg/L Sn^{2+} ; group 3: 1400 mg/L Sn^{2+}; group 4: 700 mg/L $Sn^{2+,}$ then two groups containing 1000 mg/L F^-, group 5: 2100 mg/L Sn^{2+} and group 6 Sn^{2+}: 1400 mg/L Sn^{2+}. All preparations were adjusted to pH 4.5. Enamel loss was determined profilometrically after the last experimental day. As expected, the greatest dental enamel loss µm was found in the control group. All test solutions were able to reduce tissue loss significantly ($p \leq 0.001$) but the reduction of tissue loss by test solutions depended on the ratio of the tin concentration to the fluoride concentration. It was found that the addition of high concentrations of tin 2100 mg/L Sn^{2+} to the fluoride solutions of groups 1 and 5 were very effective in reducing erosive enamel loss, and their efficacy increased with increasing concentration of tin to fluoride concentrations (Schlueter et al., 2009a).

In comparison to the present study, the therapeutic mouthrinses contained stannous and sodium fluoride in lower amounts proved highly effective in minimising the erosive and abrasive enamel surface loss. However, (Schlueter et al., 2009a) did not investigate the effect of tooth brushing which was not included in the study methodology and the erosive exposure was for a greater period and duration (5 minutes/ 6 times per day), whereas, in the present study the erosive exposure was more realistic (2 minutes/ 5 times per day).

5.6.3 Discussion of casein phosphopeptide-amorphous-calcium phosphate results

Tooth Mousse cream as a remineralising agent in studies

CPP-ACP = casein phosphopeptide-amorphous calcium phosphate; TM = Tooth Mousse.

The casein phosphopeptides (CPP) are derived from milk protein casein which has the sequence -Pse-Pse-Pse-Glu-Glu- where Pse is a phosphoseryl residue, stabilised calcium and phosphate ions in aqueous solution that make these essential nutrients bioavailable. Under alkaline conditions the calcium phosphate is present as an alkaline amorphous phase complex, referred to as casein phosphopeptide-amorphous calcium phosphate (CPP-ACP).

With the incorporation of fluoride ions, the CPP-ACP complex converts to casein phosphopeptide amorphous calcium fluoride phosphate (CPP-ACFP) and helps to stabilise the calcium. Phosphate and fluoride ions are present to provide more bioavailable and stable ions to remineralise the carious lesions in dental enamel. Hence for that reason it is considered as a safe novel product for calcium, phosphate, hydroxide and fluoride ions to provide treatments of carious lesions at early stages (Cross et al., 2004).

5.6.3.1 CPP-ACP in remineralisation studies

The systematic review of 98 articles regarding the efficacy of CPP-ACP (Azarpazhooh and Limeback, 2008) found that most of the clinical trials were focused on caries prevention. Seven studies out of nine showed that CPP-ACP was effective in reducing the caries activity in both smooth and fissure dental caries by subsurface remineralisation of carious lesions in situ in a dose-response experiment. Caseinophosphopeptides (CPP) and glycomacropeptides (GMP) have been incorporated in various oral hygiene products as anti-cariogenic agents such as Tooth Mousse and research has suggested CPP and GMP can inhibit the growth of some species of cariogenic bacteria such as *Streptococcus mutans.* Furthermore, it interacts with amorphous calcium phosphate (AMP) to form Nano-clusters at the tooth surface to provide a reservoir of calcium and phosphate ions to maintain a state of super-saturation with respect to tooth enamel (Aimutis, 2004).

A randomised, double-blind crossover remineralisation study by (Reynolds et al., 2008) conducted with 5 dentifrice slurries and an in situ appliance housing enamel slabs with subsurface lesions was conducted. The five treatments of dentifrice slurries (the addition of 1 g of paste to 4 mL de-ionised water and vortex-mixing for 60 sec) and rinsing 4 times per day for 14 days were (i) placebo, (ii) 1100 ppm Fluoride as NaF, (iii) 2800 ppm Fluoride as NaF, (iv) 2% CPP-ACP, and (v) 2% CPP-ACP plus 1100 ppm Fluoride as NaF. The formation of this CPP-ACP/F -complexes in the mouthrinse/dentifrice formulations (a mixture of CPP-APC plus 1100 ppm F toothpaste) showed that CPP increased fluoride incorporation into subsurface enamel and substantially increased remineralisation of subsurface lesions of enamel compared with fluoride alone. The dentifrice containing 2% CPP-ACP plus 1100

ppm Fluoride when compared with all other dentifrice formulations the fluoride incorporated into the lesions was significantly higher for the '2% CPP-ACP plus 1100-ppm-F' dentifrice than for the 1100 ppm F alone. Similarly the same investigator (Reynolds et al., 2008) conducted an *in situ* study with a three-way crossover randomisation involving three experimental mouthrinses. Each rinse was used for 60 sec 3 times a day; (i) 2% w/v CPP-ACP (RecaldentTM, CASRN 691364-49-5) as supplied by Recaldent Pty Ltd (Melbourne, Australia) (ii) 450 ppm F as NaF in de-ionised water; and (iii) a placebo control rinse as de-ionised water at pH 7 to assess incorporation of fluoride into dental plaque.

It was observed that both fluoride rinses produced an increase in plaque fluoride levels; the 450-ppm-fluoride rinse produced a plaque fluoride level that was nearly double that obtained with the placebo control rinse but the addition of 2% CPP-ACP to the 450-ppm-fluoride rinse significantly increased the incorporation of fluoride ions into plaque which was over double that obtained with the fluoride rinse alone. Therefore, not only did the mixture of CPP-APP with fluoride increase the incorporation of fluoride ions to subsurface caries lesions but also increased the plaque fluoride level when mixed with fluoride toothpaste (Reynolds et al., 2008).

It was proposed that under acidic conditions, this localised CPP-ACP buffered the free calcium and phosphate ions, substantially increasing the level of calcium phosphate in plaque and, therefore, maintaining a state of supersaturation that inhibited enamel demineralisation and enhanced remineralisation (Azarpazhooh and Limeback, 2008).

5.6.3.2 CPP-ACP in erosive / abrasive studies

Over the last 50 years, milk products have been tested and considered as potential anti-cariogenic agents but when considering erosion, the efficacy of GC Tooth Mousse is uncertain. This 'water-based crème' contains Recaldent™ (CPP-ACP), Tooth Mousse (GC International Tokyo, Japan).

The present study results showed there was no significant protection against five exposures of 0.3% citric acid solution erosion for 2 minutes per day and remineralisation cycling procedures with 1 hour remineralisation between acidic erosion and twice NaF toothpaste slurry brushing applied for two minutes and then rinsed with de-ionised distilled water. The application of the GC Tooth Mousse after rinsing following the last tooth brushing was applied with cotton pellets over the enamel surfaces about 2 mm thick for 5 minutes then kept overnight in night time saliva without rinsing the remineralising agent. This was performed for 28 days and the assessments were made at intervals after 7, 14, 21, and 28 days. The results could be influenced by the presence of another highly specialised formula of therapeutic products designed for protection against erosion. Group 1 contained Elmex® Sensitive Plus having 1400 ppm F and twice daily application of Elmex® erosion mouth rinse compared with Pronamel NaF toothpaste advanced formula with 5% potassium nitrate combined with Pronamel mouth wash applied twice a day for 1 minute. Furthermore the control procedure in the present study was medicated toothpaste without fluoride (non-fluoride® toothpaste, Boots, UK) aimed at daily cleaning to protect against plaque formation and refresh the mouth by adding mint freshness.

Similar results to the present study findings have been reported (Wang et al., 2011) where the investigator concluded that there was no significant protection offered by the different novel CPP-ACP pastes in an *in vitro* study in the presence of novel test agents using GC Tooth Mousse, GC MI Paste Plus (10% Recaldent™ combined with 900 ppm Fluoride (CPP-ACFP), or 7.5% w/w NovaMin®. These showed no protection before, or repair after, the erosive challenge with 25 ml of orange juice (pH 3.6) for 3 minutes and then, remineralisation for 4 hours with collected natural saliva for 3 minutes then storing in artificial saliva for 15 hours, repeated for four days. The reasons for no significant protection against erosion may be due to the precipitation from CPP-ACFP in the erosive lesion, i.e. on the tooth surface, might be generated to a limited extent and was very possibly dissolved in the following acidic attack (pH 3.6) (Wang et al. 2011).

Though, in that erosion model, despite the presence of fluoride embedded in the CCP-ACP the agents did not offer any protection, irrespective of its application before or after the erosive attack (p>0.05), which was contrary to the findings of the remineralisation *in situ* study of Reynolds et al. (2008). The differences in the structural characteristics of carious and erosive lesions may be responsible for the different nature of remineralisation in these lesions. The addition of fluoride to the CPP-ACP could be expected to strengthen the anti-erosion capability of the precipitations on the tooth surface (Wang et al., 2011). Also, it was observed that highly fluoridated AmF gel was able to protect against enamel erosion while a combination of CCP and 250 ppm fluoride provided little protection (Lennon et al., 2006).

Similarly, the present results are in agreement with the findings of Wegehaupt and Attin) (2010). Their experimental investigations also found that the application of

CPP-ACP-containing Tooth Mousse was less effective for erosion / abrasion compared to treatments of bovine enamel sections with amine/sodium fluoride gel (pH 4.8; 12,500 ppm F), sodium fluoride gel (pH 7.1; 12,500 ppm F). Samples in group 1 remained untreated and served as negative controls. The test samples were treated for 2 min/day as follows: group 2 amine/sodium fluoride gel (pH 4.8; 12,500 ppm F), group 3 sodium fluoride gel (pH 7.1; 12,500 ppm F) and group 4 CPP-ACP-containing Tooth Mousse and were subjected to de- and remineralisation cycling performed for 20 days with 6 erosive attacks for 20 s with HCl (pH 3.0) per day. Samples were stored in artificial saliva between cycles and overnight. Tooth brushing abrasion was performed with (15 s; 60 strokes/min; load 2.5 N) with toothpaste slurry performed each day before the first and 1 h after the last erosive exposure. Compared to baseline surface profiles, the corresponding post-treatment profiles revealed significant tooth wear reduction for the amine/sodium fluoride gel (pH 4.8; 12,500 ppm F), while the treatment with CPP-ACP containing Tooth Mousse was less effective and did not significantly reduce the erosive / abrasive wear compared to the control (Wegehaupt and Attin, 2010).

An *In vitro* experiment (White et al., 2011) revealed that the specimens treated with distilled water (DIW)(negative control) displayed a 58.2% reduction in surface hardness. Three solutions statistically significantly inhibited softening: 0.5% w/v casein (21.8% reduction in surface hardness; $p \leq 0.005$), 300 ppm F (13.3%; $p \leq 0.003$) and 0.5% w/v casein + 300 ppm F $p < 0.050$). The other solutions had no statistically significant impact on softening in comparison to the DIW control: CPP (50.8% without 300 ppm F, 48.2% with) and GMP (62.4% without 300 ppm F had 66.1%.

There were also no significant differences between the application of a tooth cream containing 5% casein/calcium phosphate and the untreated control specimens on

bovine enamel specimens for 120 seconds twice daily. They found no significant difference with respect to erosive enamel loss (bovine enamel specimens rinsed with artificial saliva and erosive challenge using 1% citric acid (pH 2.3) for 30 seconds six times daily for 14 days when compared with the untreated control group after seven and 14 days (Lennon et al., 2006).

Conversely, in an *in vitro* study it was found that a single topical application of Pronamel or Tooth Mousse would prevent enamel erosion in a group of human enamel samples that were treated with either Pronamel or Tooth Mousse applied for 15 min. The control group was placed in distilled water for 15 min, after exposure of all specimens to an erosive challenge of 0.2% citric acid for 1h. The results for the Pronamel group were statistically significantly different from the control group at the $p<0.01$ and the results for the Tooth Mousse group were statistically significantly different from the control group at the $p<0.05$ level and concluded that: Tooth Mousse and Pronamel may offer a degree of protection from erosion of permanent enamel (Rees et al., 2007). In this erosive model it was observed that Pronamel might protect erosive enamel by about 48% and using Tooth Mousse by about 35%. However that study did not simulate normal everyday life practices and the enamel specimens were exposed for longer erosive periods in 0.2% citric acid followed by a single application of Pronamel or Tooth Mousse for 15 minutes. This did not involve tooth brushing abrasion, whereas, in the present study, the group of enamel slabs consisted of 15 specimens subjected to twice daily mechanical tooth brushing with Pronamel toothpaste first (15 strokes) and then a single application of Tooth Mousse once a day for 5 minutes at the end of each experimental day for 14 days cycling for an extended duration and the assessment was made at intervals after 7, 14, 21, and 28 days.

5.7 Conclusion

1. The twice daily topical application of (Elmex® Sensitive Plus (AmF) toothpaste plus Elmex® erosion mouthrinse) and Sensodyne Pronamel® toothpaste and mouthrinse combinations demonstrated a highly significant effect against acidic enamel erosion and tooth brushing abrasion procedures compared to fluoride-free toothpaste (control).
2. The effectiveness of using two combinations of the twice daily topical applications of either (Elmex® Sensitive Plus (AmF) toothpaste plus Elmex® erosion mouthrinse) or Sensodyne Pronamel® toothpaste and mouthrinse had a prominent significant effect on enamel surface loss subjected to 0.3% citric acidic erosion and tooth brushing abrasion compared to a single application of remineralising agent (GCTooth Mousse).
3. There was no significance between the remineralising agent (GC Tooth Mousse) and fluoride-free toothpaste on reducing the enamel surface loss subjected to both acidic erosion and tooth brushing abrasion.

5.8 Recommendations

It is suggested that a regimen comprising a combination of (Elmex® Sensitive Plus (AmF) toothpaste plus Elmex® erosion mouthrinse) or Sensodyne Pronamel® toothpaste and mouthrinse products might be more beneficial in providing better protection against repeated acidic erosion and tooth brushing abrasion than non-fluoridated toothpaste or combined with a remineralising agent.

Further *in situ* studies are needed to verify the effectiveness of fluoridated toothpastes alone and in combination with other specific oral hygiene products.

6 Study 3: Investigations of therapeutic products on prevention of enamel surface loss under erosive and abrasive challenges *in situ*

6.1 Introduction

The erosion process involves demineralisation and remineralisation periods of slabs that cause dissolution of the tooth surface (Eccles, 1978; Linnett and Seow, 2001). Dissolution of mineralised tooth structure occurs upon contact with acids that are introduced into the oral cavity from intrinsic (e.g., gastro-oesophageal reflux, vomiting) or extrinsic sources (e.g., acidic beverages, citrus fruits). Enamel softening occurs due to partial demineralisation. Theoretically at this stage, the presence of fluoride causes surface remineralisation (Lussi et al., 2006).

Increased demineralisation of the surface creates two layers, surface and subsurface layers. If the surface layer is totally demineralised repair is not possible as the bulk tissue has been lost. Surface or sub-surface layers of partially demineralised tissue may be remineralised (Jones et al., 2002; Lussi et al., 2004a). The interest in dental erosion has increased dramatically over the last ten years. This increased interest is due to the decrease in incidence of dental caries and the increased interest of scientists in dental erosion (Lussi et al., 2004b).

Tooth wear (tooth surface loss) is recognised as a major problem in both children and adults (Nunn et al., 2003; Lussi et al., 2006). The triad of erosion, attrition and abrasion has been known for many years but the contribution of erosion to tooth wear may be increasing. Dental erosion is the irreversible loss of dental hard

tissue due to a chemical process of acid dissolution but not involving bacterial plaque acid and not directly associated with mechanical or traumatic factors or with dental caries. Variables that affect dental erosion include pH; temperature and exposure time (Amaechi et al., 1999c; Eisenburger and Addy, 2001); titratable acidity and buffering capacity (Lussi et al., 1993; Larsen and Nyvad, 1999); salivary pellicles (Meurman and Frank, 1991; Amaechi et al., 1999d); remineralisation effect of saliva (Kelly and Smith, 1988; Amaechi and Higham, 2001; Attin et al., 2003); abrasion (Attin et al., 1998; Jaeggi and Lussi, 1999) and fluoride (Boulton et al., 1997; Attin et al., 1999). Studies that have investigated the role of fluoride on the reduction or prevention of dental erosion used different in vitro protocols to produce erosive lesions (Ganss et al., 2001b; Ganss et al., 2004b; Fowler et al., 2006) and different fluoride products i.e. fluoride acidified gel (Attin et al., 1999; Jones et al., 2002), mouth rinse (Lussi et al., 2004a) and toothpaste (Eisenburger and Addy, 2001; Hooper et al., 2007).

In a study by Lussi and co-workers (2004a) that used two different mouthrinses (250ppm F as sodium fluoride and amine fluoride, and 250ppm F as amine fluoride) *in situ* as a single treatment and found that there was no effect of tooth brushing abrasion combined with these mouth rinses. Currently, no standard protocol exists for investigating erosive / abrasive procedures. It is common (1) to apply the demineralising agent for an unrealistically long time period (e.g. 30 min). However various laboratory investigations in dental erosion are focusing on exposure of tooth substrates to acidic exposure or combined with tooth brushing but the erosion process is more complex and the biological factors are playing a significant role in the mechanism of erosion (Zero and Lussi, 2000).

6.2 Study synopses

6.2.1 Aim

To investigate different therapeutic products on the prevention and treatment of enamel surface loss under daily citric acid erosive and tooth brushing abrasive challenges using a longitudinal *in situ* model.

6.2.2 Objectives

6.2.2.1 Primary objective***:***

To evaluate the effects of fluoridated products alone and in combination on surface loss of bovine enamel compared with a placebo in an experimental *in situ* longitudinal erosion model.

6.2.2.2 Secondary objectives***:***

1. To verify the treatment effects of fluoridated oral products within the groups.

2. To compare the results of this study to previous *in situ* and *in vitro* studies.

3. To use the results of this study in the development of methodology for future erosive / abrasive *in situ* studies and to obtain data to aid in power calculations for future studies.

6.2.3 Null hypothesises

1. There are no differences in the effectiveness of using two combinations of (Elmex® erosion toothpaste plus Elmex® erosion mouth rinse or Sensodyne Pronamel® toothpaste plus Sensodyne Pronamel® mouthwash) on enamel surface loss subjected to both 0.3% acidic erosion (pH 3.6) and tooth brushing abrasion compared to a single application of Elmex® erosion toothpaste or Sensodyne Pronamel® toothpaste.

2. There are no differences in the effectiveness of using two combinations of topical applications of (Elmex® erosion toothpaste plus Elmex® erosion mouth rinse or Sensodyne Pronamel® toothpaste plus Sensodyne Pronamel® mouthwash) on enamel surface loss subjected to both 0.3% acidic erosion (pH 3.6) and tooth brushing abrasion compared to non-fluoride® toothpaste.
3. There are no differences in the effectiveness of using Elmex® erosion toothpaste or Sensodyne Pronamel® toothpaste compared to non-fluoride® toothpaste.

6.3 Study plan

6.3.1 Flow chart

Recruitment

↓

Screening visit

(Signed consent form, salivary flow rate, DMFS score)

↓

Preparation of in situ appliances

Wash-out period (2 days)

↓

Experimental phase (14 days) for 2 minutes application of the toothpaste /or 1 minute use of mouth wash and immersion in 0.3% citric acid 2 minutes / 5 times per day as per instruction sheet

The subjects record the exact times of dipping on a diary card.

↓

The slabs were removed for analysis with Surface Profilometry after 14 days and new fresh slabs were attached to the oral appliance.

↓

Wash-out period (2 days)

↓

Repeat with each experimental oral product

↓

Final dental check

6.3.2 Study schedule

Steps	Visit 1 Screen And Taking Impression	visit 2 Day 1	visit 3 Day 3 Start 1st phase	visit 4 Day 17 End 1st phase	visit 5 Day 20 Start 2nd phase	visit 6 Day 34 End 2nd Phase	visit 7 Day 38 Start 3rd phase	visit 8 Day 52 End 3rd phase	visit 9 Day 55 Start 4th phase	visit 10 Day 69 End 4th phase	visit 11 Day 72 Start 5th phase	visit 12 Day 86 End 5th phase	visit 13 Day 87 Final Dental Check
Consent	X												
Medical History	X												
Demographics	X												
Concurrent Medications	X												
Inclusion/Exclusion	X												
Oral Soft and Hard Tissue Exam	X	X	X	X	X	X	X	X	X	X	X	X	
Salivary flow rate	X												
Continuance Criteria		X	X	X	X	X	X	X	X	X	X	X	
Partial Denture		X	X		X		X		X		X		
Distribute Washout		X		X		X		X		X		X	
Collect Washout			X		X		X		X		X		
Place Slabs			X		X		X		X		X		
Collect Slabs				X		X		X		X		X	
Randomisation		X											
Distribute Product/Diary			X		X		X		X		X		
Collect Product/Diary				X		X		X		X		X	
Supervise Brushing		X	X		X		X		X		X		
Non-Treatment Events	X	X											
Adverse Events			X	X	X	X	X	X	X	X	X	X	
Dental Check and													X

6.3.3 Ethical considerations

Before conducting this in situ clinical trial a series of ethical approval processes were followed, revised and obtained by the Ethics Research Committee (REC), Research & Development Department (R & D) and also Site Specific Permission (NHS SSP).

Study approval numbers

1- REC number (11/YH/0367) Appendix 10

2- NHS permission at LTHT number (DT 11/ 10039) Appendix 11

This single centre, 5 phases, controlled, double-blind, crossover, *in situ* study was conducted in accordance with the Declaration of Helsinki (1964) and to the guidelines of Good Clinical Practice (2000).

The guidelines of The Consolidated Standards of Reporting Trials (CONSORT 2010) was also followed strictly (Moher et al., 2010) Figure 6.1. Signed, dated and witnessed consent forms were obtained from all participants before enrolling into the study after adequate verbal and written explanations of the study. All participants had the right to withdraw at any time during the study (Appendices 12 & 13).

6.3.3.1 CONSORT (2010) Flow Diagram

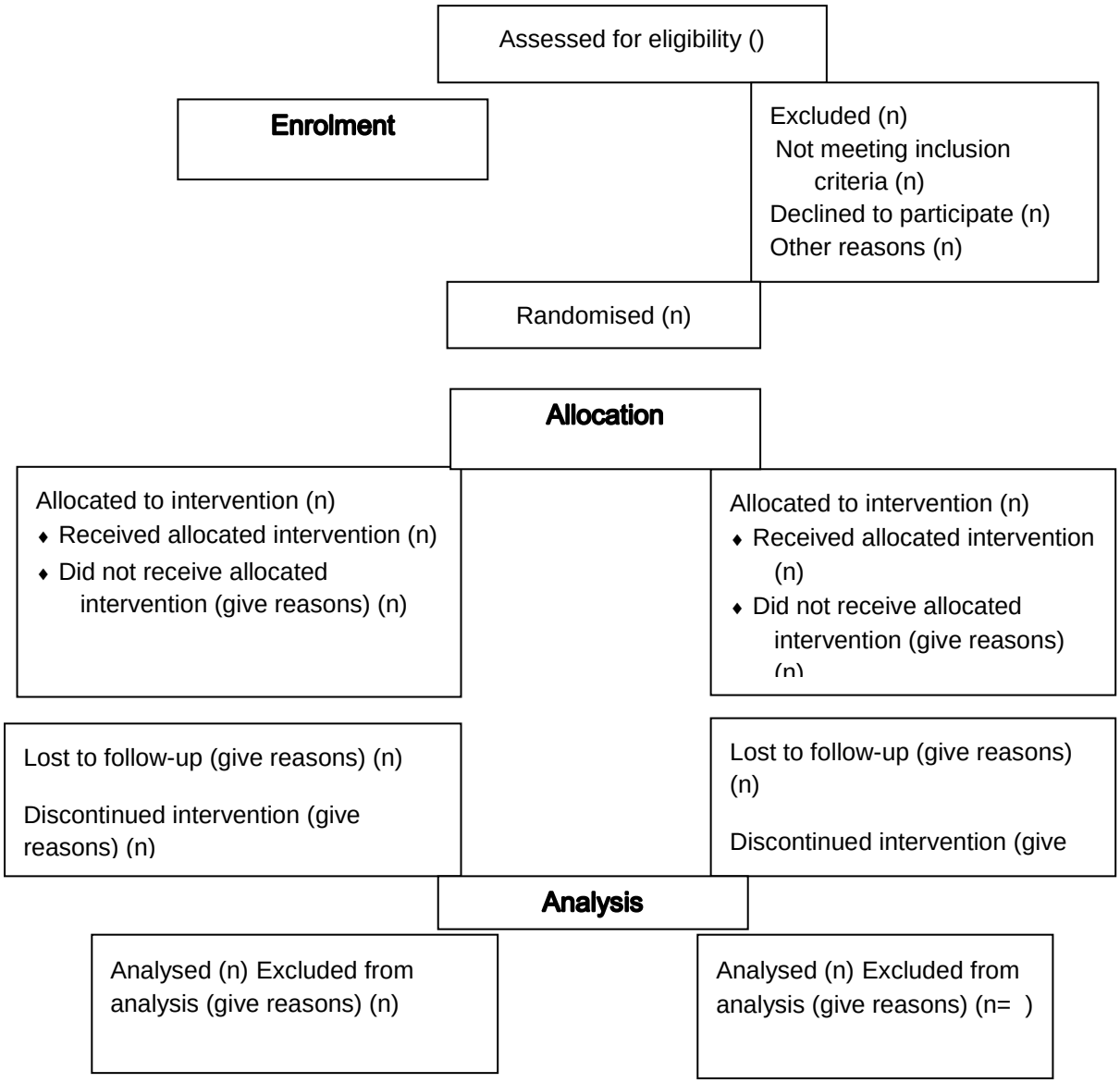


Figure 6.1 COSORT 2010

(Courtesy of Moher et al., 2010)

The total participants assessed and enrolled for eligiability was (n=20), three of them were withdrawn.

6.4 Study products

6.4.1 Experimental standard fluoride toothpastes and mouth wash combinations

Fluoridated toothpastes and mouth wash combinations as follows:

1. Sensodyne Pronamel® toothpaste (GlaxoSmithKline, UK).
2. Pronamel® toothpaste (GlaxoSmithKline, UK) plus Sensodyne Pronamel® anti-erosion mouth wash (GlaxoSmithKline, UK).
3. Elmex® erosion protection toothpaste (GABA International Switzerland).
4. Elmex® erosion protection toothpaste (GABA International Switzerland) with Elmex® erosion protection dental rinse (GABA International Switzerland).

6.4.2 Control Product

6.4.2.1 0 ppm F toothpaste (non-fluoride®, Boots, PLC Nottingham England).

6.5 Study duration and timings

As described above, the study had five phases (study arms) with each phase lasting two weeks. There were two day wash-out periods between the study arms. The clinical trial took place from 12th June 2012 until 29th November 2012.

6.6 Criteria for evaluation

The earlier preliminary work showed that measuring the amount of surface loss was an ideal technique to compare the effect of different preventive toothpastes. Therefore, the mean values of the surface loss (µm) were used as a method of comparison. The amount of surface loss was calculated using light surface profilometry (Proscan Scantron 2000) which measured the depth of the eroded

surface in relation to intact surfaces with the help of attached automated software supplied with the surface profilometer, and then, the difference between treatment groups was determined.

6.7 Study design

This was a randomised, controlled, double-blinded, cross-over study. The study included 5 independent phases (study arms), each to test one of the experimental products. The length of each phase was 14 days. Volunteers wore an oral palatal appliance during the working day from 9:00 am till 17:00 pm, except when they were eating, drinking or applying study products and overnight. Any product put in the mouth, including breath and mouth fresheners was considered as eating and drinking. The subjects made every attempt to avoid leaving the appliance out of the mouth for extended periods of time (i.e. greater than 1 hour). However, if the subject should leave the appliance for more than one hour outside the mouth, then the subject was instructed to insert the appliance into the mouth for at least 5 minutes every hour that the appliance was to be left out of the mouth.

The subjects were asked to report each of these events in their diary cards. In addition, subjects were not permitted to chew gum during the experimental study procedures, i.e. from the start of the first wash-out period through to the follow-up visit. The amount of surface loss (µm) was calculated using a light surface profilometer (Proscan Scantron 2000, UK) which measured the depth of the eroded surface in relation to the reference intact surfaces. The amount of surface loss was calculated by using the automated software supplied with the surface profilometer (Proscan 2000, UK).

6.7.1 Rational for study design

This model had been used previously by this research group. Our previous *in vitro* work showed that after at least 14 days of cycling using this model, comparable results could be detected. Therefore, the length of each phase was set at 14 days.

Volunteers wore a removable upper palatal appliance to comply with the standard position of a similar device used in previous in situ studies. This had been approved by the Bristol Healthcare Trust Ethical Committee and the palatal site was chosen for volunteer acceptability and with the expectation that the enamel slabs would be contacted by the oral rinse (West et al., 1998, Hara et al., 2009a). A regimen of wearing an intra-oral palatal appliance fitted with samples from 9:00 am to 17:00 pm and storing the device in a moistened condition during the night has been validated (West et al., 1998, Hunter et al., 2000, Hooper et al., 2005, Hooper et al., 2007, Messias et al., 2010). In order to avoid the contact of the erosive solution with the subject's teeth, dipping of the intra-oral removable appliance with the attached enamel samples in the supplied 0.3% citric acid solution was performed out of the mouth in a given plastic dipping box for 2 minutes/ 5 times per day. Then, the oral appliance and the enamel samples were rinsed with tap water before re-insertion in the mouth.

In this present study, the effect of erosive and tooth brushing abrasion procedures were studied against the effect of toothpastes/and or in combination with mouth rinses. Surface profilometry was used to measure the amount of surface loss and then to compare between groups.

6.7.2 The source of dental material and ethical considerations

Permanent incisors were obtained from young cattle at a local abattoir. Permission for the collection of bovine teeth was obtained from the Meat Hygiene Service (approval No. 2091) granted for the Dispatch of SRM for Veterinary or Research Purposes. Certain animal health measures were taken in UK to eradicate bovine spongiform encephalopathy (BSE) among cattle since 1988 and government agencies continued to implement measures to minimise the risk (Brown et al., 2001).

The teeth were transported to the laboratory in 0.1% thymol solution. In the laboratory they were decoronated on a diamond band saw. The crowns were cleaned to remove stains with fluoride-free pumice, washed and stored in 0.1% thymol solution in a tightly sealed container at 4°C until required.

6.7.3 The rational use of bovine enamel for evaluation of dental material in the present in situ model

Bovine enamel specimens have been used widely in recent dental research as a safe and good substitute for human enamel (Ruse et al., 1990, Lagerweij et al., 2006, Rios et al., 2006, Rios et al., 2008a). Bovine enamel has a number of advantages required for certain simple and straightforward methods, such as surface microhardness (Vieira et al., 2005) and represents a reproducible model for erosion experiments (Nekrashevych and Stösser, 2003). Human enamel is becoming increasingly difficult to obtain and is of a highly variable composition when compared to bovine enamel (Mellberg et al., 1992, Fontana et al., 2004).

6.7.4 Bovine enamel in *in situ* studies

Bovine enamel slabs have been widely used in various in situ models as a safe and good substitute for human enamel (Rugg-Gunn et al., 1998, Turssi et al., 2004, Magalhaes et al., 2008c, Hara et al., 2009a, Hara et al., 2009b, Messias et al., 2010, Okunseri et al., 2011).

Participants were fully informed about the source of the enamel specimens because it might conflict with certain beliefs or religions.

6.7.5 Study population

Twelve females and five males participated in a randomised crossover design study. The age ranges of the volunteers were from 23 to 55 years old. They were mainly from the staff at Leeds Dental Institute or students in Leeds University and were recruited via electronic mail or advertisements in the postgraduate room and dental hospital. Persons interested in the study were given comprehensive oral explanations and information sheets (Appendix 12). After signing an informed consent (Appendix 13), subjects were asked about their general health and were evaluated according to the inclusion and exclusion criteria.

They were given a dental examination before the start of the study to determine their DMFT/DMFS using BASCoD criteria. Statistical data from the present study research group were used to calculate the sample size for this study. Statistical assistance was sought from the departmental statistician using sample size power calculation from previous published material (Magalhaes et al., 2008b). The outcome was measuring the enamel source loss for each volunteer and calculating the mean value, 5 treatments, 5 periods and independent phases,

duration of each treatment = 14 days in subjects over 18 years wearing intra-oral appliances holding 2 enamel slabs. In order to allow comparisons within the groups the effect size = minimum difference/standard deviation from previous published material. Using the software for power analysis and sample size calculation and a planned minimum clinical difference $\alpha = 0.05$Using p-=0.05, a (5) delta(1.34) alpha(0.05) rho (0.6). At least 18 subjects were needed to get a reasonable estimate in order to yield enough data with a power of 89%. It was planned to include 20 subjects in the study to allow for any subsequent withdrawal during the study.

6.7.5.1 Inclusion criteria

1. Adults with normal salivary function, who were not taking medication that, could affect the salivary flow rate or oral pH. Subjects were asked to sit quietly and drool into a disposable volumetric tube for five minutes in order to establish the salivary flow rate. A minimum salivary flow rate of 0.25ml/min was required for participation in the study.

2. Minimum of 18 natural teeth.

3. Free from clinical signs of periodontal disease.

4. Compliant.

5. Medical history did not include any medical contra-indications like epilepsy, risk of infective endocarditis, haemophilia, or pregnant/ nursing subjects.

6. Provided written informed consent, authorisation for the release of health information for research and medical history information prior to their participation;

7. Aged from *18*-65 years old and in good health with no evidence of communicable diseases;

8. An unstimulated whole salivary flow rate ≥ 0.25 ml/min and a stimulated whole salivary flow rate ≥ 0.8 ml/min;

9. Should be able to wear the appliances –at specified hours as required by the protocol.

10. Should be able to comply with the experimental procedures.

6.7.5.2 Exclusion criteria

1. Signed informed consent not obtained by the volunteers.
2. Adults who were taking drugs that affect salivary flow rate.
3. Volunteers with complex medical histories (e.g. epileptic subjects, subjects at risk of infective endocarditis, or pregnant/nursing subjects).
4. Volunteers who regularly used erosive products, i.e. vitamin C or fizzy drinks.
5. Volunteers who had a course of antibiotics in the previous 4 weeks.
6. Volunteers who had antimicrobial treatment in the previous 2 weeks.
7. Volunteers with complex dental histories such as periodontitis, rampant caries or salivary dysfunction.
8. Volunteers with allergies to any of the materials used in the study.
9. Had medical conditions that could be expected to interfere with the subject's safety during the study period.

10. Taking any medication that could potentially react with the study test products.

11. Required antibiotics prior to dental treatment.

12. Demonstrated an inability to comply with study procedures.

13. Wearing removable prosthesis and orthodontic appliances.

6.7.5.3 Subject withdrawal criteria

Subjects had the right to withdraw from the study at any time for any reason. The investigator also had the right to withdraw subjects from the study in the event of development of illness, adverse events, treatment failure after a prescribed procedure, protocol deviations, administrative reasons or other reasons. It was understood by all concerned that an excessive rate of withdrawals could affect the study power calculation; therefore, unnecessary withdrawal of subjects should be avoided. Should a subject decide to withdraw, all efforts should be made to complete and report the observations as thoroughly as possible. A complete final evaluation at the time of the subject's withdrawal should be made with an explanation of why the subject was withdrawing from the study.

If the reason for removal of a subject from the study was an adverse event or an abnormal laboratory test result, the principal specific event or test should also be recorded on the case report form '(CRF)'. A description of the 'stopping rules' or 'discontinuation criteria' for individual subjects should be described.

6.7.5.4 Subject replacement

If a subject discontinued before completing all study assessments, a replacement subject was entered into the study. The replacement subject was screened and underwent all the investigations as per the study protocol for treatment phases.

6.7.6 Study Treatment Supplies Management

6.7.6.1 Experimental commercially available oral products were:

1. Sensodyne Pronamel® 1450 ppm F as NaF toothpaste.
2. Sensodyne Pronamel® 1450 ppm F as NaF toothpaste plus Pronamel® mouth wash as 450 ppm F NaF.
3. Elmex® erosion protection toothpaste (1400 ppm F as AmF).
4. Elmex® erosion protection toothpaste (1400 ppm F as AmF) with Elmex® erosion protection dental rinse as AmF and NaF 500 ppm F plus stannous chloride 800 ppm F stannous.
5. Non-fluoride®, Boots, UK, toothpaste as a control.

In addition, each volunteer was supplied with 14 pre-weighed plastic bottles of citric acid (each contained 1.5 mg) and 500 ml graduated plastic bottles to produce a fresh erosive solution every day (1.5 mg of citric acid mixed with 500 ml tap water).

6.7.6.2 Packaging and labelling

The test toothpastes were packaged with white covers and labelled using the codes supplied by the study coordinator. All study labels had to include at least the following information: study number (11/YH/0367), randomisation and period number, storage conditions, and emergency contact details. A new test tooth

brush (Macleans, medium, interdental, GSK, UK) was supplied to each volunteer at the beginning of each phase. Citric acid crystals were supplied in 7 ml plastic containers (7 ml BIJOU container, SLS SELECT LTD, Nottingham). The weight of citric acid in each bottle (1.5 mg) was enough for one day. Another 500 ml plastic bottle was supplied to mix the tap water with citric acid crystals. The bottle had an indication line to the level for the level of water to be added. In addition, volunteers were provided with a dipping pot to dip the removable appliance extra-orally in citric acid. This pot had an indication line to standardise the amount of erosive citric acid the volunteers had used for each dipping.

6.7.6.3 Accountability of study supplies

The study investigator was responsible for keeping records of all supplies to allow:

1. Identification of the subject to whom the study treatment supplies were dispensed.

2. Date and quantity of the study treatment supplies dispensed to the subject.

3. Date and quantity of the study treatment supplies returned by the subject (if applicable): The study treatment supplies were verified by the supervisor of the study at the end of the study. Each therapeutic product was measured carefully using a balance in the Dental Clinical Research Unit at the beginning and at the end of each phase (Appendix 18, Table 8.20).

6.7.6.4 Date and quantity of the study treatment returned by the subject (if applicable)

The investigator and study co-ordinator were present during the participant visits and the clinical trial manager was also available during the study monitor visits.

At the end of the study, the study treatment supplies were verified by the supervisor of the study.

6.7.7 Enamel slab preparation

Similar slab preparation procedures were followed as described in sections 3.3.1, 3.3.2, 3.3.3.1, 3.3.3.2 and 3.3.3.4 except for covering with nail varnish and mounting the slabs in an acrylic *in vitro* plate.

6.7.7.1 Baseline measurements

Knoop microhardness (KMH) was used as an inclusion criterion for the enamel slabs, which were used in the study. This inclusion criterion was used as recommended by our preliminary *in vitro* work since it reduced the outliers and standardised the hardness of the slabs (as described in section 3.3.3.5.2).

Baseline measurements were recorded using KMH as an inclusion criterion. Microhardness was assessed using a computer-aided Duramin Indenter Machine (Struers A/S, DK 26-10, Denmark). The indentations were created using a Knoop diamond under a 100 g load for 15 seconds for enamel (Zero et al., 1990, Panich and Poolthong, 2009). The length of indenter penetration was measured by means of an image analysis system. Three KMH indentations were created for each slab and the mean was determined. The length of each indent was recorded and the mean of the three indentations was calculated (Figure 6.2).

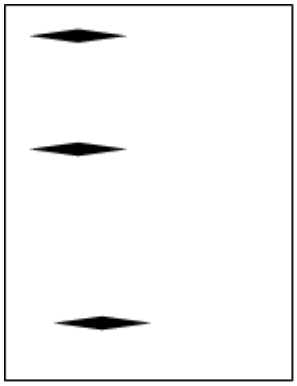


Figure 6.2: baseline indentation measurements

6.7.7.2 Surface profilometery (SP)

SP provided an idea of the surface profile of the slabs that were used in this study. In addition, it measured the depth of surface loss. Therefore, SP was used at the beginning of the study to ensure that the slab' surfaces were flat and at the end of each phase of the study to measure the depth of surface loss (µm) calculated in relation to two unexposed reference areas.

6.7.7.3 Storage of the slabs

Once the slabs had been prepared, they were kept moist in de-ionised distilled water in micro-centrifuge tubes at room temperature until sterilised.

6.7.7.4 Sterilisation and storage of enamel slabs

The enamel slabs were stored damp in sealed containers and exposed to gamma radiation (4080 Gy) as an effective method for enamel sterilisation in an *in situ* model as no significant changes in enamel hardness are observed. This level of exposure has been shown to give sterilisation without altering the structural integrity of the enamel. Greater exposure to gamma irradiation also affects the demineralisation and remineralisation characteristics of the enamel hardness

(Chandler, 1990, White et al., 1994, Pollard, 1995, Büyükyilmaz et al., 1997a, Amaechi et al., 1998b, Duggal et al., 2001, Zero et al., 2006, Viana et al., 2010). Then the slabs were immersed in 2.5% sodium hypochlorite for 1 hour to eliminate prions (Taylor, 1991, Taylor et al., 1994, Taylor, 1999). A previous study showed that 5% sodium hypochlorite did not have an effect on the mineral content of dentine or its crystal structure (Driscoll et al., 2002). After treatment, the slabs were placed in de-ionised water until they were analysed. The enamel slabs were handled at all times using disposable medical gloves.

6.7.8 Experimental appliance

A maxillary removable palatal appliance with U clasps on the upper first permanent molars and acrylic plate on the palatal surface was made for each volunteer. Two enamel slabs were secured in the palatal plate of the appliance. The slabs were attached to the side of the midline and secured with sticky wax; care was taken to ensure that the wax did not cover the exposed surfaces of the slabs. The slabs were exposed to the oral environment but were protected from the effect of the tongue using arched wires leaving a space of 1 mm between the wire and the slabs. This design of partial denture is commonly used for children to prevent them from thumb sucking; therefore this design will have had no effect on the volunteers (Figures 6.2 and 6.3).

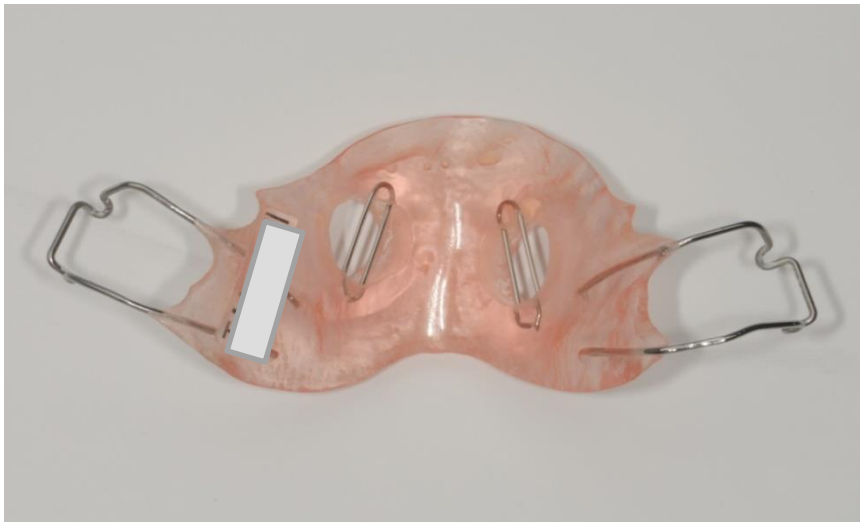


Figure 6.2 Shows the upper removable in situ acrylic intra-oral appliance with two holes with inserted two slabs covered with two arched protective wires to prevent the effect of the tongue on the slabs

(The two reference areas were covered with light cure composite resin (3M ESPE, Filtek ^{TM} Z250 USA) without etch or bonding agent leaving the middle experimental area unexposed. Composite resin was applied on the reference areas of the enamel surfaces in order to comply with the high standard hygienic procedures.

Figure 6.3 Orientation of the wire over the hole made in the appliance

Courtesy from Abdullah et al. 2009.

6.7.9 Blindness and Randomisation

6.7.9.1 Blindness

The analysis with Profilometry was carried out blindly, without the investigator knowing the origin of the enamel slabs to ensure blindness. In addition, the test materials were coded and the codes were kept with the study coordinator. Neither

the principal investigator nor volunteers knew the codes of the oral products during the study. In order to comply with the double blind study, the two study mouthrinse bottles were re-dispensed into 500 ml plastic containers (500ml white HDPE bottle with fitted white HDPE tamper-evident screw cap, sterile irrad; Medfor) which were opaque with identical shapes and capacities (400 ml each).

6.7.9.2 Rules for breaking the study blindness

The blindness would only be broken in an emergency where it was essential to know which treatment a subject received in order to give appropriate medical care. The investigator would sign and date the broken code envelope and give the reason for breaking the code.

6.7.9.3 Randomisation

Following the baseline evaluations, the subjects meeting all the eligibility criteria were randomised and were given one of each of the five treatments at each study period according to a randomisation schedule (Appendix 14 Table 8.19).

6.7.10 Study procedures and assessments

6.7.10.1 Place of examination (study site)

The Dental Clinical Research Unit with its highly advanced and equipped facilities is situated on level 5 at Leeds Dental Institute (Figure 6.4). In that unit all infection control procedures were strictly followed and all the guidelines and regulations of Good Clinical Practice. Participants were under the care of well experienced examiners (MD, JT and FA), clinical trial manager (CF) who was monitoring the trial

procedures to ensure safety measures and a highly qualified study coordinator (GD) and her co-assistant (AC) were involved in this controlled clinical trial.

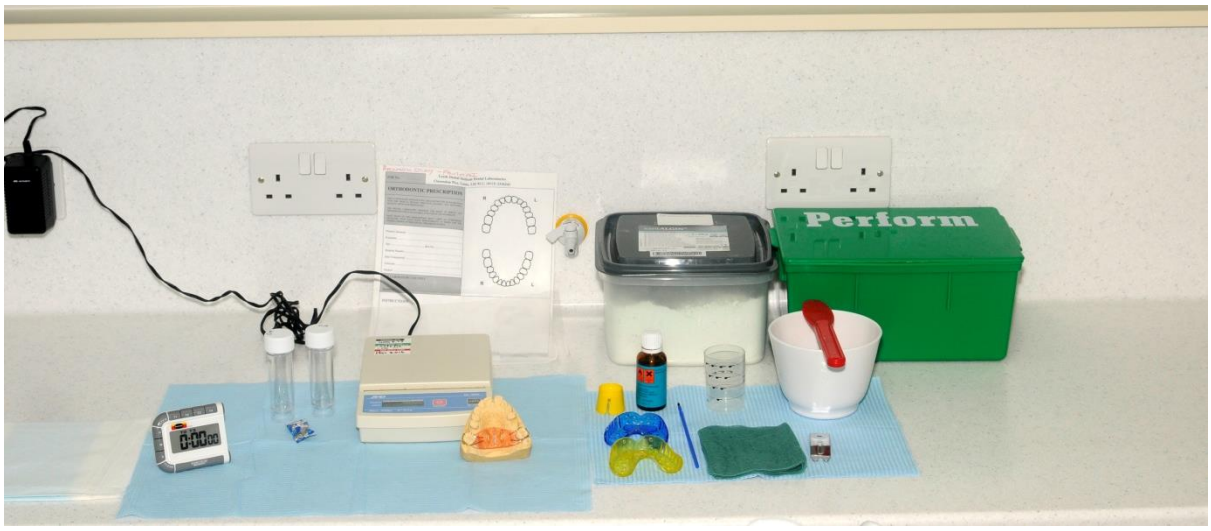


Figure 6.4 Pre-adjustment and organised dental materials before arrival of the participant in the Clinical Trial Research Unit (Leeds Dental Institute).

6.7.10.2 Informed consent

Prior to commencing any study related activity, the investigator obtained witnessed written (signed and dated by the subject) informed consent from each individual participating in this study after adequate explanation of the aims, methods, objectives, and potential hazards of the study (Appendix 13). The investigator explained to the subjects that they were completely free to refuse to enter the study or to withdraw from it at any time. Appropriate forms for the investigator provided documenting a written consent. The investigator recorded the date and time of the consent in the subject's records. Any subject was considered to be enrolled into the study after the informed consent had been signed and witnessed.

6.7.10.3 Screening

A subject screening record and a case record form (CRF) were used to document the screening evaluation along with any reason for failure. Potential subjects were selected from a panel of volunteers at the study site (Appendix 17). The subjects were asked to visit the study site and were provided with information regarding the purpose and the conduct of the study, both verbally and in writing. They were given time to decide if they wished to participate. If they decided to proceed they were asked to provide a written consent to participate in the study. Those subjects who consented to the study were selected at the study site (Fig. 6.4) for a screening visit and were instructed to refrain from any oral hygiene for 48 hours prior to the visit.

At the screening visit, eligible subjects entered into a washout period as they refrained from using their toothpaste and they used the non-fluoride toothpaste provided by study investigator. Then, they were randomised to receive the study products and a CRF was completed for all randomised subjects. Information for subjects who were enrolled but not randomised was captured in a screening log.

The following evaluations were performed during the screening visit:

1. Demographics.

2. Medical history.

3. Concomitant medication.

4. Review of inclusion/exclusion criteria.

5. Oral examination included a thorough examination of the oral soft tissue status and DMFS measurement. This examination was repeated at each visit during the

study.

6. Salivary flow rate.

7. Impressions made for *in-situ* oral appliances.

8. Subjects who successfully fulfilled all the necessary entrance criteria were provided with a standard toothbrush. This was dispensed to the subject at the screening visit.

6.7.10.4 Treatment phase

6.7.10.4.1 Washout Period

A washout period of at least 2 days prior to the start of study phase one was commenced after the screening visit. The washout period between study levels had a duration of 2 days. During the washout periods, the subject was only permitted to use the standard toothbrush and the non-fluoride® toothpaste (Boots, England, UK), and abstained from all oral hygiene procedures (flossing and using a breath freshener or mouth wash etc.). At the end of each study phase, the subject left the *in situ* intra-oral appliance at the study site so that it could be disinfected and fitted with two new fresh slabs to be inserted into the appliance in preparation for the next study phase.

6.7.10.4.2 Acclimatisation period

Prolonged use of the *in-situ* oral appliances can cause discomfort to the subject. Hence, a period of acclimatisation with the *in-situ* oral appliance of 2 days was conducted prior to commencing phase one (i.e. concurrent with washout period of

study phase 1). During this period subjects asked to wear the appliance at all times (except when eating, drinking, or brushing their teeth).

For this study any product put in the mouth, including chewing gum, flossing or using a breath freshener or mouthwash would be considered as eating and drinking. If the subject experienced any discomfort, they were asked to return to the study site for the appliance to be adjusted. Then, they were advised to continue with the acclimatisation period.

6.7.10.4.3 Follow-up period

Subjects attended a follow-up visit within 14 days of the final assessment day. This visit could also occur at the same time as the last study treatment phase. The visit included a brief medical interview, oral examination and optional application of commercially available topical fluoride gel if necessary.

6.7.11 Screening and baseline measurements and evaluations

6.7.11.1 Screening

6.7.11.1.1 Demography

The investigator recorded each subject's date of birth, gender and race in the CRF (Appendix 17).

6.7.11.1.2 Medical history

The investigator took the medical history of each subject including details of any relevant medical or surgical history, allergies or drug sensitivity. The Investigator (or medically qualified designee) also reported in the CRF details of any concomitant medications. Additionally, the investigator (or medically qualified designee) reviewed the inclusion/exclusion criteria and ensured the subject's

eligibility to enter the study. Any concomitant medication taken by the subject within 30 days of screening through to study conclusion were reported in the CRF by the investigator or study co-ordinator. Both current and concomitant medications were restricted in accordance with the exclusion criteria.

6.7.11.2 Oral examination and DMFS measurement

At the start of the study the subject was given a dental examination to determine the DMFT/DMFS, using BASCoD criteria by a well experienced examiner. The results of the oral examination were recorded in the CRF as either normal or abnormal with any abnormalities being described.

6.7.11.2.1 Salivary flow rate

The subjects were seated in a quiet, comfortable position, with their head tilted forward so that saliva collected at the front of the mouth. The subject was asked to swallow to clear their mouth of any residual saliva. This action marked the start of a 5-minute saliva collection period. During this 5-minute period, the subject was not permitted to swallow any saliva but required to spit or dribble any excess saliva into a graduated collection bottle to measure the salivary flow rate.

For the stimulated collection, subjects chewed on gum base (paraffin gum) for one minute. After one minute, subjects were instructed to swallow any pooled saliva. They then chewed the gum base for two minutes, timed, during which time they emptied any pooled saliva into a collection tube. During the saliva collection period the subject was not permitted to drink, chew or speak. An audible alarm was sounded after 5 minutes to indicate the end of the saliva collection period. Subjects

were required to spit all remaining saliva collection into the saliva collection bottle for measurement.

6.7.11.3 Impressions made for in situ oral appliances

Subjects were seated in a comfortable position in a dental chair. In order to protect clothing subjects were provided with a bib. Subjects used a dental mouth rinse to keep the oral cavity clean during the impression procedure.

A colour coded transparent impression tray (Polytray Dentply) was chosen to fit each subject. The chosen tray was then filled with an alginate impression material (Xantalgin select) and placed in the subject's mouth to obtain an impression of the upper and lower jaw/arch. The impressions were disinfected in Perform-ID (Schulke & Mayr) before transporting to the lab.

6.7.11.4 Experimental protocol/ regime

The subjects were assigned to one of the five experimental regimes using specially designed appliances fixed with two enamel slabs. The regime is shown in Table 6.1.

Table 6.1 Experimental protocol / regime

1st period	2 days	Washout
	2 weeks	Dipping the appliance for 2 min, morning and evening into Pronamel® toothpaste as 1450 ppm F NaF. During the day, the patient will dip the appliance 2 minutes / 5 times into a 0.3% citric acid extra-orally.
2nd period	2 days	Washout
	2 weeks	Dipping the appliance for 2 min, morning and evening into Pronamel® toothpaste as 1450 ppm F NaF plus Pronamel® mouth wash for 1 minute/twice a day. During the day, the patient had to dip the appliance 2 minutes / 5 times into a 0.3% citric acid extra-orally.
3rd period	2 days	Washout
	2 weeks	Dipping the appliance for 2 min, morning and evening into Elmex® toothpaste 1400 ppm F as AmF. During the day, the patient had to dip the appliance 2 minutes / 5 times into a 0.3% citric acid extra-orally.
4th period	2 days	Washout
	2 weeks	Dipping the appliance for 2 min, morning and evening into Elmex® toothpaste as AmF 1400 ppm F and rinsing with Elmex® erosion protection dental rinse twice/day for 1 min. During the day, the patient had to dip the appliance 2 minutes / 5 times into a 0.3% citric acid extra-orally.
5th period	2 days	Washout
	2 weeks	Dipping the appliance for 2 min, morning and evening into 0ppm F toothpaste. During the day, the patient had to dip the appliance 2 minutes / 5 times into a 0.3% citric acid extra-orally.

The appliances were worn at specified times by the volunteers from 9.00 am till 17.00 pm daily, except at mealtimes, whilst drinking, or during tooth brushing and

overnight. Dipping into toothpaste was achieved by asking the volunteers to brush for 1 minute using the toothpaste supplied as normal and while the appliance was out of the mouth. Then, the volunteers inserted the appliances in their mouth before rinsing with water and they were asked to swish using the toothpaste in their mouth for 1 minute. After that, volunteers rinsed with water as normal.

The oral rinse was performed by the volunteer with 10 ml of the allocated solution for 60 seconds while the oral appliance was inside the mouth. The appliances and the attached enamel slabs were maintained moist in a sealed plastic container when removed from the mouth and overnight. The enamel slabs were collected at the end of each period and the volunteers were supplied with new slabs at the beginning of each phase. The volunteers were asked to use fluoride-free toothpaste provided for them twice daily during the 2 days of washout period. The full trial therefore lasted 3-4 months.

Volunteers were given supplies for two weeks at each visit. Therefore, volunteers were asked to attend the study site for each leg as follows:

1. 1st Visit: screening and taking measurements for appliance fitting.
2. 2nd Visit (at d**a**y 1): to check the fit of appliance and (washout period).
3. 3rd Visit (at day 3): to give supplies for 1st study arm and to check the appliance.
4. 4th Visit (at day 17): to collect dipping diary and appliance (washout period).
5. 5th visit (at day 20): to give supplies for the 2rd study arm.
6. 6th Visit (at day 34): to collect dipping diary and appliance (washout period).
7. 7th Visit (at day 38): to give supplies for 3rd study arm.
8. 8th Visit (at day 52): to collect dipping diary and appliance (washout period).

9. 9th Visit (at day 55): to give supplies for 4th study arm..

10. 10th Visit (at day 69): to collect dipping diary and appliance (washout period).

11. 11th Visit (at day 72): to give supplies for 5th study arm.

12. 12th Visit (at day 86): to collect dipping diary and appliance.

13. 13th Visit (at day 87): final dental check and oral prophylaxis.

6.7.11.5 Compliance

Volunteers' compliance was checked using the following methods:

1. Each volunteer was provided with a study diary to monitor and record each step during the study (Appendix 16).

2. The study diary was checked during volunteers' visits.

3. Collecting the used citric acid bottles and measuring the remnants of each bottle and record that this was appropriately disposed.

4. Collecting used toothpaste tubes and mouthrinse bottles and measuring the used amounts (Appendix 18).

5. Collecting the used toothbrushes after each study Phase and checking the bristles.

6.7.11.6 Adverse events

All adverse events (adverse experiences/adverse drug experiences) encountered during the clinical study, whether spontaneously reported by the subject at any time during the study or elicited by the investigator in a standard manner at the study visits, were reported in the CRF.

The investigator or study co-ordinator asked the subject the following question during each visit including any follow-up visits: “Have you felt unwell, experienced any symptoms or taken any medication *(since your last visit) (today) (since your last dose) (since the last session).”*

All adverse events encountered during the clinical study were reported on the CRF. An Adverse Event (AE) was any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and which did not necessarily have to have a causal relationship with this treatment. An AE could therefore be any unfavourable and unintended sign (including an abnormal laboratory finding, for example), symptom, or disease temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product. Furthermore, an AE could be any unintended change (including physical, psychological or behavioural) from the subject’s baseline (pre-treatment condition), including intercurrent illness, which occurred during the course of a clinical trial after treatment had started, whether considered related to treatment or not. “Treatment” includes all investigational agents (including placebo) administered during the course of the study. Changes associated with normal growth and development not varying in frequency or magnitude from that ordinarily anticipated clinically were not adverse events (e.g., onset of menstruation occurring at a physiologically appropriate time).

Clinical adverse events were described by diagnosis and not by symptoms whenever possible (e.g., cold, seasonal allergies, etc. instead of runny nose).

An overdose was a deliberate or inadvertent administration of a treatment at a dose higher than specified in the protocol and higher than known therapeutic

doses. It must be reported irrespective of outcome even if toxic effects were not observed.

Adverse events were graded on a three-point scale and reported in detail as indicated on the CRF:

Mild - easily tolerated, causing minimal discomfort and not interfering with normal everyday activities.

Moderate - sufficiently discomforting to interfere with normal everyday activities.

Severe - incapacitating and/or prevents normal everyday activities.

Trial study relationship for each adverse event was determined by the investigator using the following explanations:

Not related – The event was clearly related to other factors such as the subject's clinical state, therapeutic interventions, or concomitant medications administered to the subject.

Unlikely – The event was most likely produced by other factors such as the subject's clinical state, therapeutic interventions, or concomitant medications administered to the subject; and did not follow a known response pattern to the trial drug.

Possible – The event followed a reasonable temporal sequence from the time of drug administration; and/or followed a known response pattern to the trial drug; but could have been produced by other factors such as the subject's clinical state, therapeutic interventions, or concomitant medications administered to the subject.

Probable – The event followed a reasonable temporal sequence from the time of drug administration; and followed a known response pattern to the trial drug; and could not be reasonably explained by other factors such as the subject's clinical state, therapeutic interventions, or concomitant medications administered to the subject.

Highly Probable – The event followed a reasonable temporal sequence from the time of drug administration; and followed a known response pattern to the trial drug; and could not be reasonably explained by other factors such as the subject's clinical state, therapeutic interventions, or concomitant medications administered to the subject; and either occurred immediately following trial drug administration, or improved on stopping the drug, or reappeared on repeat exposure, or there was a positive reaction at the application site.

The subject's self-management of adverse events (graded as moderate or severe) was assessed by the investigator at each visit as appropriate or not.

Any adverse events on-going at the follow-up visit, which had any association with the study medication or the study regime were followed up until resolved by the study site and for two weeks after the subject's last visit by the sponsor, if resolution did not occur sooner. Any resolutions confirmed by the study site were noted on file and a copy provided for the sponsor's records. If any event was not resolved before completion of the final report, the report would be issued and an addendum would be generated detailing the resolution of the event.

6.7.11.7 Serious adverse events

Any clinical adverse event, including abnormal laboratory test value, that is serious (as defined below) occurring during the course of the study, irrespective of the treatment received by the subject, must be reported to the sponsor within 24 hours (or sooner if possible) of the investigator or designee becoming aware of the situation.

A serious adverse event is any adverse experience occurring at any dose that results in any of the following outcomes:

Death

Life threatening (places the patient, in the view of the initial reporter, at immediate risk of death from the adverse experience as it occurred, i.e., it does not include an adverse experience that, had it occurred in a more severe form, might have caused death).

Persistent or significant disability/incapacity (disability is a substantial disruption of a person's ability to conduct normal life functions); in-patient hospitalisation or prolongation of hospitalisation; congenital anomaly/birth defect;

Important medical events that may not result in death, be life-threatening, or require hospitalisation may be considered a serious adverse experience when, based upon appropriate medical judgment, they may jeopardise the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in the definition.

Pregnancy is not considered to be a serious adverse event but must be reported on a Confidential Pregnancy Experience Form provided.

The term ‘severe’ is a measure of intensity; thus a severe adverse event is not necessarily serious. For example, nausea of several hours duration may be rated as severe, but may not be clinically serious.

A death occurring during the study or which comes to the attention of the investigator within 4 weeks after stopping the treatment whether considered treatment-related or not, must be reported.

For all serious adverse events, the investigator must inform the sponsor’s clinical operations by telephone within 24 hours or sooner if possible, of becoming aware of the situation. Subsequently the serious adverse event must be assessed for the following details: date of onset, date ceased, frequency, intensity, action taken regarding test substance, treatment required for experience, relationship to test substance, is event serious and outcome to date. These details must be recorded on the clinical study Serious Adverse Event Form that is provided in the investigator study master file. This form should be transmitted by fax and the details given by telephone to the investigation site. Unless otherwise directed, the CRA will copy details of all SAEs to the Medical Director as well as the Medical Safety Group.

Weekends, holidays, and after 5 p.m. call:

If there is no reply, record details on the message service.

Such preliminary reports will be followed by detailed descriptions later which will include copies of hospital case reports, autopsy reports and other documents when requested and applicable.

REC should be notified of such an event in writing as soon as is practical.

6.7.12 Statistical methods and analytical plan

6.7.12.1 Demographic and baseline characteristics

Information of randomised subjects, number of evaluable subjects, age, gender and race was collected. Amount of tooth surface loss (hard tissue findings) were recorded. Descriptive statistics for continuous measures included the number of subjects, mean, median, standard deviations and minimum/maximum.

6.7.12.2 Efficacy

6.7.12.2.1 Primary efficacy variables

The mean tooth surface loss (µm) change was calculated using the automated software of profilometry within each enamel block. The comparison between the test products was conducted using the appropriate SPSS analysis. The significance level initially was set at 0.05.

The primary analysis population for the efficacy variables was calculated to detect a difference of 5 µm between the two groups. This figure was used after considering the previous in vitro results. In addition, the possibility of remineralisation of the dental slabs in the oral environment was considered. The power calculation was set to be 90%.

6.7.12.2.2 Secondary efficacy variables

For multiple treatment effect comparisons within the groups, a p value was further adjusted and the differences were considered significant when ($\alpha \leq 0.005$).

6.7.13 Monitoring of the study

The monitoring of the study was the responsibility of the supervisor, both the chief and the principal investigators Professor. They regularly conducted meetings with the investigator and inspected the various records of the study (CRFs and other pertinent data), provided that subject confidentiality and blindness were maintained.

6.7.14 Study documentation, CRFs, and record keeping

6.7.14.1 Investigator's files/retention of documents

The investigator maintained adequate and accurate records to enable the conduct of the study to be fully documented and the study data to be subsequently verified. These documents were classified into two different separate categories (1) investigator's study master file, and (2) study/subject clinical source documents.

6.7.14.2 Case Report Forms (CRFs)

For each subject who gave an informed consent, a CRF was completed and signed by the principal investigator to certify that the data within each CRF were complete and correct. This was also applied to those subjects who failed to complete the study prior to randomisation. If a subject was withdrawn from the study because of a treatment-limiting adverse event, thorough efforts were made to document the outcome.

All forms were filled out using a black ball-point pen during (or immediately after) a subject assessment, and were legible. Errors were crossed out, but not obliterated or covered with correction fluid, the correction inserted, and the change initialled and dated by the investigator and the study co-ordinator.

CRFs were reviewed by the study monitor at the study site. Errors detected by subsequent in-house CRF review were clarified or corrected. All changes were documented and approved by the investigator.

6.7.15 Assessment

6.7.15.1 Surface profilometer (SP)

Baseline measurements of the surface profile of the slabs were assessed using the Surface Profilometer (Scantron ProScan 2000) to ensure that the average height to the average depth range was ± 1.0 µm. The measurement was achieved by placing the sample on a key stage on the Scantron ProScan and using a 150 mm height of the camera as standard. The step size was 0.01 mm. After scanning, the average height to the average depth range of the surface was measured. This could be achieved using the automated software mentioned by defining the areas of interest. The enamel slabs' surfaces were then covered with composite resin (3M ESPE, Filtek ^{TM} Z250 USA) without using acid etch or bonding agent except for a small window in the middle of each slab. After the test period the composite resin was removed using a dental flat plastic instrument and the same procedure was repeated to check the depth of surface loss (µm).

6.8 Results

6.8.1 Data handling

The primary parameter for surface profilometery was the depth of surface loss that was defined as the change from the start of the treatment period to the end of treatment. Comparisons between baseline and treatment were verified. Comparisons between groups were made using the appropriate parametric / non-parametric statistics. A significance level of $p \leq 0.05$ was accepted.

6.8.1 Statistical analysis

In order to comply with "blindness" during the study, the statistical analysis was performed before breaking of the blindness.

6.8.1.1 Distribution of enamel surface loss changes during the experimental phases

Table 6.2 shows that there were no missing enamel slabs detected among the study groups (34 enamel slabs were present in each phase) Appendix 19. The product B (non-fluoride toothpaste) (had the highest erosive and abrasive enamel surface loss value (µm) (mean ± standard deviation) which was (13.75 ± 4.71µm) and the lowest amount of enamel surface loss recorded was for treatment group E (Elmex erosion® protection toothpaste plus mouthrinse) which was 3.015 ± 2.39 µm). The next lowest amount of enamel surface loss was recorded for treatment D (Sensodyne Pronamel® toothpaste plus mouthrinse) was 4.78 ± 2.87 µm). However the treatment by product A (Elmex® erosion protection toothpaste) had mean values of (6.03 ± 4.71 µm) and

product C (Sensodyne Pronamel® toothpaste) had values of enamel surface loss which were (6.55 ± 5.91 µm).

Table 6.2 Case summaries of the distribution of mean values (µm) median, minimum and maximum values and the total number of the analysed enamel slabs in the experimental groups

Treatment	Number of slabs	Mean	Maximum	Median	Minimum	Std. Deviation
Elmex® erosion protection toothpaste (AmF, 1400 ppm F)	34	6.03	20.6	5.2	.59	4.71
Non-fluoride® toothpaste (0 ppm F)	34	13.8	48.5	8.6	1.48	12.6
Sensodyne Pronamel® toothpaste (NaF, 1450 ppm F)	34	6.55	23.7	4.5	1.0	5.91
Sensodyne Pronamel® toothpaste plus Sensodyne Pronamel® mouthrinse	34	4.78	10.1	4.2	1.05	2.87
Elmex® erosion protection plus Elmex erosion® mouthrinse	34	3.02	10.7	2.2	.54	2.39

6.8.1.2 Distribution of mean values of the tested therapeutic products

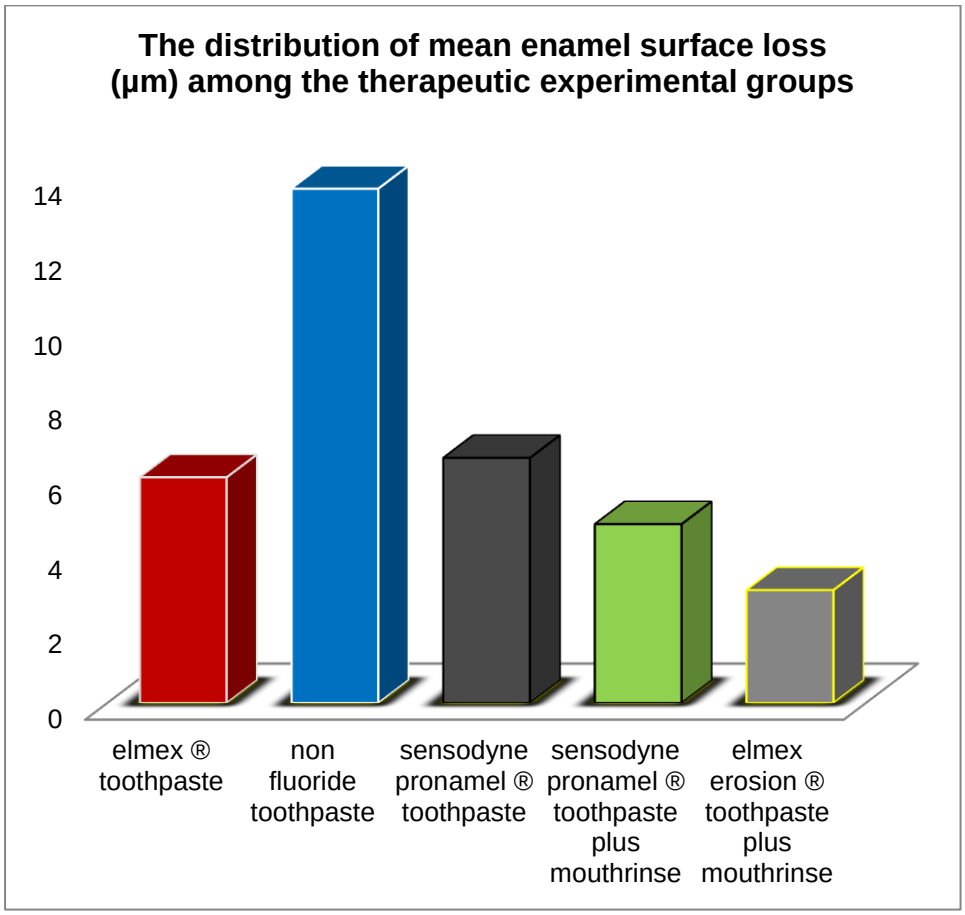
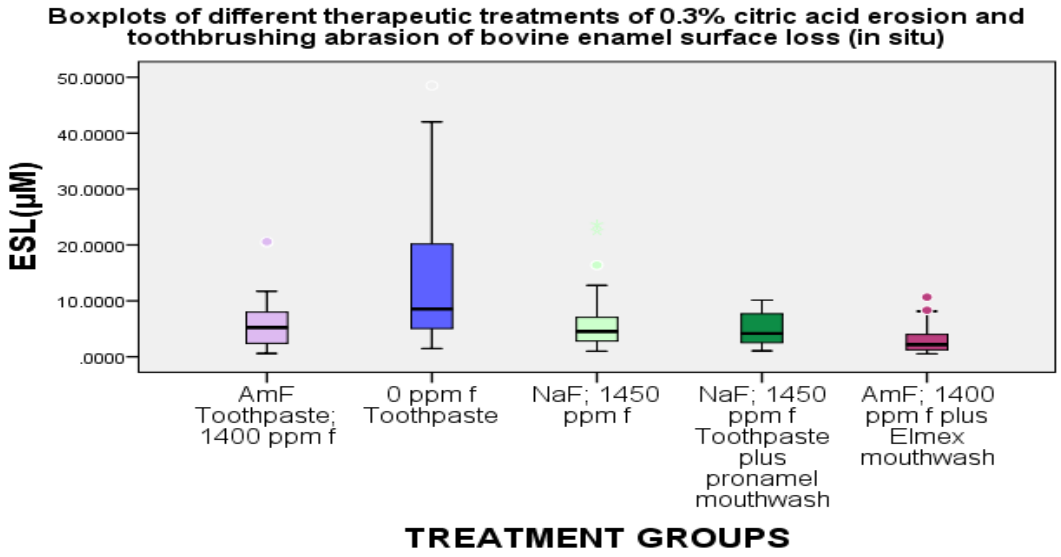


Figure 6.5 Distributionn of different changes of enamel surface loss values (µm) among the experimental groups caused by 0.3% acidic erosion and twice daily tooth brushing during the in situ study.

Figure 6.5 show that the non-fluoride group had the highest mean surface loss (µm) in contrast to the other groups. The lowest mean surface loss was noticed for groups that were treated with both Elmex® erosion protection toothpaste (Elmex toothpaste) and Elmex erosion mouth rinse. However, Elmex® erosion protection toothpaste, Sensodyne Pronamel® toothpaste and combined Sensodyne Pronamel® toothpaste and Pronamel mouthrinse demonstrated nearly similar trends of enamel surface loss (µm) values under 0.3% citric acid erosion and twice daily tooth brushing or combined with twice daily rinsing with the allocated mouth rinse.

6.8.1.3 Enamel surface loss changes (µm) of the tested therapeutic products in the in situ model

Boxplots display median, upper and lower quartile changes in 0.3% citric acid erosion and tooth brushing abrasion among the treatment groups at the end of crossover randomised phases (Figure 6.6).



*** and o are outliers and extremes values.**

Figure 6.6 Enamel surface loss changes (µm) of all therapeutic experimental groups caused using 0.3% citric acid erosion and twice daily tooth brushing during the *in situ* crossover study for 14 days.

The box-and–whisker plots display the median, maximum and minimum values, in addition to the first and third quartiles within the experimental measurements. The horizontal line within each box of the box-and–whisker plot represents the median value for each group of the experimental treatments.

The median change for the non-fluoride® toothpaste product was higher than for all other groups, whereas the median change in the Elmex® erosion protection toothpaste plus mouthrinse group was the lowest compared to the other treatment groups. However, products Elmex® erosion protection toothpaste, sensodyne Pronamel® toothpaste and Sensodyne Pronamel® toothpaste plus mouthrinse showed approximately similar median changes.

The outliers were identified as O and * within the treatment groups in which one outliers was present in the Elmex® erosion protection toothpaste group. Three outliers were present in the Sensodyne Pronamel® toothpaste plus mouthrinse group and two in the Elmex® erosion toothpaste plus mouthrinse group (Figure 6.6).

6.8.1.4 Model for cross-over design

Taking into consideration the complex interaction of factors incorporated within the erosive/abrasive in situ models regarding treatments, periods, the persons and the cross-over design that was used for this study, a mixed effects model (Jones and Kenward, 2003, Diaz-Uriarte, 2002) was used to analyse the data in this study. The equation of the mixed model that was used is shown below:

$$\gamma ijk = \mu + Sik + \pi j + \tau d + e\text{ij}k$$

Where $\gamma\text{ij}k$ is the enamel loss for subject i period j and treatment k, μ is the intercept, Sik is the treatment effect for subject i at period k, πj is the period effect and τd is a random subject effect, $e\text{ij}k$ is the error term for subject I, period j and treatment k (in order to transfer the equation into a word document).

In this *in situ* cross-over study, the model that was applied was as follows:

Erosive/ abrasive enamel loss = intercept + period effects + treatment effects + random subject effect + error (refers to any other effect not explained in this study).

Table 6.3: represents mixed effects (intercept, treatment, subject and the period/phase) of the cross-over study showing the different effective criteria that had influence.

6.8.1.5 ANOVA results of tests for between-subjects effects

Table 6.3 ANOVA test results between subjects 'effects

	Type III Sum of Squares	df	Mean Square	p value
Intercept	4781.4	1	4781.4	.000
Treatment	996.3	4	249.1	.000
Person	2596.9	16	162.3	.000
Phase	770.5	4	192.6	.000
Phase * Treatment	1285.7	16	80.4	.000

The results in Table 6.3 show that the main effect of treatment, phase and the interaction of phase by treatment was statistically significant. Since the treatment by phase was significant $p \leq 0.005$., so that the null hypothesis was rejected) Table 6.3).

The two-way outcomes (means for treatment by phase) are shown in (Table 6.4).

6.8.1.6 The distribution of the means for treatment by phase (2x2 design table)

This 2x2 table also demonstrates the method of supply of the treatment products during the randomised phases and how many slabs were included during that particular phase (Table 6.4).

Table 6.4: Distribution of the means for treatment by phase in (2x2 design)

Treatment	Phase	Number of slabs	Mean
Elmex® erosion protection toothpaste	1	4	1.43
	2	6	4.9
	3	8	7.7
	4	10	7.7
	5	6	5.6
Total		**34**	**6.03**
Treatment	Phase	Number of slabs	Mean
Non-fluoride toothpaste	1	8	11.2
	2	10	10.3
	3	2	6.7
	4	8	11.0
	5	6	28.9
Total		**34**	**13.75**
Treatment	Phase	Number of slabs	Mean
Sensodyne Pronamel® toothpaste	1	6	3.8
	2	8	5.3
	3	2	1.5
	4	12	6.0
	5	6	13.7
Total		**34**	**6.55**
Treatment	Phase	Number of slabs	Mean
Sensodyne Pronamel® Toothpaste and mouthrinse	1	12	6.0
	2	2	1.5
	3	8	4.6
	4	2	4.5
	5	10	4.1
Total		**34**	**4.8**
Treatment	Phase	Number of slabs	Mean
Elmex® erosion protection Toothpaste and mouthrinse	1	4	1.64
	2	10	5.04
	3	12	2.79
	4	2	1.21
	5	6	1.60
Total		**34**	**3.02**
Phase 1		34	5.80
Phase 2		36	6.33
Phase 3		32	4.6
Phase 4		34	7.30
Phase 5		34	10.0
Total		170	6.82

6.8.1.7 Multiple comparisons between the therapeutic products

A Bonferroni test correction test was applied at a higher level and the p value was adjusted at $p \leq 0.005$. Pair-wise comparisons and the dependant variable are shown in Table 6.5.

As shown in Table 6.5:

1. The treatment by therapeutic products Elmex® erosion protection toothpaste, Sensodyne Pronamel® toothpaste, Sensodyne Pronamel® toothpaste plus mouthrinse and Elmex® erosion toothpaste and rinse demonstrated highly statistical significant levels in reducing the erosive and abrasive enamel surface loss ($p \leq 0.001$) compared to treatment with non-fluoride toothpaste.
2. Treatment by non-fluoride® toothpaste showed the least statistical significant effect in decreasing the erosive and abrasive enamel wear compared to all other treatment groups.
3. The Elmex® erosion protection toothpaste and Elmex® erosion protection rinse had the greatest statistical value in reducing the erosive and abrasive enamel wear compared with non-fluoride® toothpaste and Sensodyne Pronamel® toothpaste ($p \leq .001$).
4. There was no statistical significant difference observed between Sensodyne Pronamel® toothpaste plus Sensodyne Pronamel® mouthrinse and Elmex® erosion protection toothpaste and Elmex® erosion protection rinse $p > 0.05$.
5. The Elmex® erosion protection toothpaste and Elmex® erosion protection rinse had statistical significant effect than Elmex® erosion protection toothpaste alone $p \leq 0.01$.

Table 6.4 Comparisons between the five therapeutic test products on enamel surface loss (µm) changes achieved using 0.3% citric acid erosion and twice daily tooth brushing abrasion during 14 days of an *in situ* crossover study.

Treatment groups pair-wise comparisons		Mean Differ-ence	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Elmex® erosion protection toothpaste	Non-fluoride toothpaste	-7.72*	1.08	.000	-9.86	-5.58
	Sensodyne Pronamel® Toothpaste and mouthrinse	1.25	1.08	.250	-.891	3.39
Non-fluoride® toothpaste	Sensodyne Pronamel® toothpaste	7.20*	1.08	.000	5.06	9.34
	Sensodyne Pronamel® Toothpaste and mouthrinse	8.97*	1.08	.000	6.83	11.11
	Elmex® erosion protection Toothpaste and mouthrinse	10.73*	1.08	.000	8.59	12.87
Sensodyne Pronamel® toothpaste	Sensodyne Pronamel® Toothpaste and mouthrinse	1.77	1.08	.104	-.369	3.92
	Elmex® protection erosionToothpaste and mouthrinse	3.53*	1.08	.001	1.39	5.68
Sensodyne Pronamel® Toothpaste and mouthrinse	Elmex® erosion protection Toothpaste and mouthrinse	1.76	1.08	.106	-.38	3.90
Elmex® erosion Toothpaste and mouthrinse	Sensodyne Pronamel® toothpaste	-3.53*	1.08	.001	-5.68	-1.39
	Sensodyne Pronamel® Toothpaste and mouthrinse	-1.76	1.08	.106	-3.90	.380

*
Mean difference value significant at the 0.05 level.

6.8.1.8 Profile plots of the interaction of treatments during the cross-over phase

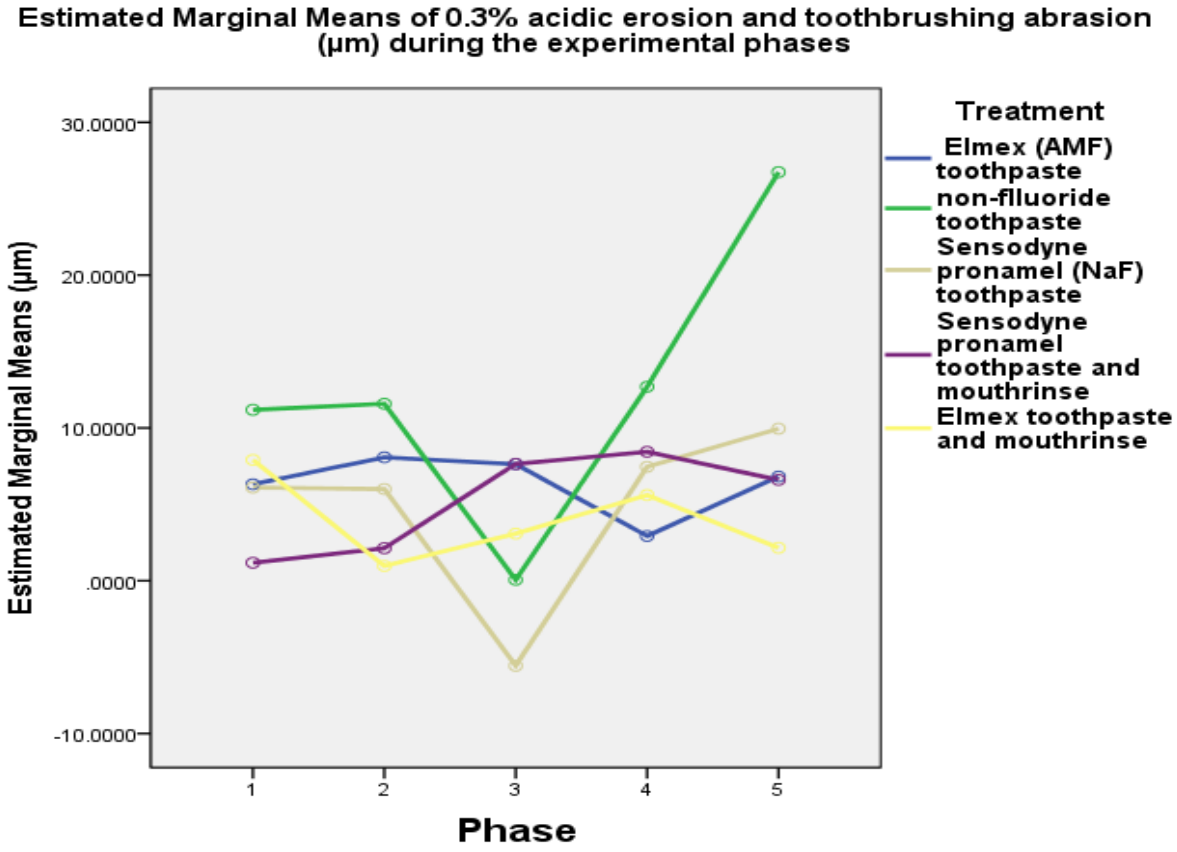


Figure 6.7 The interaction of treatments during the in situ crossover five treatment phases showing the estimated mean values (µm).

This profile plot shows the interaction of treatment where;

Elmex® erosion protection toothpaste, non-fluoride® toothpaste, Sensodyne Pronamel® toothpaste, Sensodyne toothpaste, Sensodyne Pronamel® combinations (toothpaste and mouthrinse) and Elmex® erosion combinations (toothpaste and mouthrinse).As shown in Figure 6.7 the non–fluoride toothpaste had the greatest determined marginal mean change during the cross-over phases, whereas treatments with the other therapeutic products such as Elmex® erosion protection, Sensodyne Pronamel®, Sensodyne Pronamel® toothpaste plus mouthrinse and combined Elmex® erosion protection toothpaste and mouthrinse demonstrated slight marginal changes between treatments.

6.9 Discussion

The aim of the present in situ study was to investigate the use of some therapeutic oral products that were formulated specifically for treatment of dental erosion under real-life conditions against acidic erosion and tooth brushing abrasion using either a single toothpaste or in combination with a mouthrinse. Various *In situ* studies showed that with repeated daily use of fluoride agents such as toothpastes, fluoride rinses, gels and varnishes, salivary fluoride levels increased from baseline levels as a result of fluoride accumulation in the plaque, oral tissues and saliva (Zero et al., 1992). Administering fluoride mouthrinse led to a greater increase in salivary fluoride levels than that after applying a fluoride toothpaste treatment. The results of that study indicated that the method of F delivery (fluoride gel, fluoride rinse or fluoride dentifrice), the F concentration of the agent, and the time of application (daytime or during night-time) were important factors influencing F levels in the mouth. While, the recycling of F in ductal saliva as a consequence of ingestion of home-use fluoride products did not appear to make a clinically significant contribution to F levels in the mouth (Zero et al., 1992).

Treatments with fluoride gel (1.1% as NaF) led to a significantly higher fluoride concentration than fluoride rinse or fluoride toothpaste, meanwhile the fluoride rinse with 0.05% NaF had a greater concentration than the toothpaste (0.24% NaF) after two hours of individual samplings (Zero et al., 1992). Based on the study of Zero and co-workers(1988b) it was observed that fluoride rinse with 225 ppm F as NaF was more effective in delivering fluoride in the oral cavity than fluoride toothpaste (1100 ppm F as NaF).

Owing to the universal presence of fluorides in various different forms in nature or in pharmaceutical products such as tablets, rinses, gels and toothpastes these fluoride agents have been introduced to promote human oral health as an integral part of general health promotion and quality of life (Murray, 1986, Petersen, 2008a, Petersen, 2008b). To promote a successful public health measure through "Solving Health Problems Through Global Cooperation", much research is required to ensure the continued, effective, and safe use of fluorides and exposure to the vast majority of fluoride-containing oral products (e.g. dentifrices, supplements) which are among the effective fluoride sources to ensure public exposure of fluorides to control and prevent oral diseases (Hardwick et al., 2000, Petersen, 2008a).

Prolonged exposure of the teeth to dietary acids increased if this was associated with a swishing or holding habit that might contribute to the development of erosion in some children. Moreover, children with erosion also drank milk or water significantly less often than the control groups and those with erosion were found to be more frequently taking acidic beverages, fruits, vinegar consumption and vitamin C supplements (O'Sullivan and Curzon, 2000).

The frequent use of fluoride gel and fluoride mouthrinse in addition to fluoride toothpaste offers the opportunity to minimise abrasion of tooth substance (Lussi et al., 2006). Proteolytic enzymes may act via both breakdown of the pellicle and the organic material of the enamel making them more prone to physical and chemical damage (Westergaard et al., 2001) so overall, the results showed that the various occupational and lifestyle factors might be relevant in the pathogenesis of dental erosion. Proteolytic enzymes (Westergaard et al., 1993, Westergaard et al., 2001) have also been hypothesised to occasionally cause erosion, which are frequently

seen among lacto-vegetarians, are caused by acids derived from low pH foodstuffs, but it must be emphasised that lacto-vegetarians also frequently consume coarse fresh foods, more so than controls, which adds to the tooth tissue wear.

As the critical pH of dental enamel is approximately 5.5, any solution with a lower pH value may cause erosion, particularly if the exposure is of long duration, and repeated over time. Saliva and salivary pellicle counteract the acid attacks but if the challenge is severe, a total destruction of tooth tissue follows (Meurman and ten Cate, 1996). The critical pH below which enamel dissolves is not constant but rather is inversely relative to the amount of saturation of calcium and phosphate in the saliva and plaque fluid. Teeth with early subsurface carious lesions can be remineralised, but teeth that have suffered acid erosion cannot (Dawes, 2003).

It was clearly shown in an *In situ* study that individuals vary in the amount of erosion experienced with the same acid exposure (Hall et al., 1999).

6.9.1 Rational use of the present abrasive and erosive in situ model

There is no consensus standard for *in situ* methodological models, though various dental erosion studies are available in the literature. However, standard procedures for abrasive and erosive *in situ* studies have been established (Shellis et al., 2011). There is diversity in the methodological designs of the erosive and abrasive models among researchers, and perhaps, this may be related to the complexity and nature of the different life styles and the severity of the pathological occurrences.

The monitoring of erosive and abrasive enamel wear treatments in the present study that were recorded by quantification of dental enamel loss in relation to two intact reference areas after the end of the experimental periods using non-contact laser scanning profilometry has also been proved to be a good methodological method for

both *in vitro* and *in situ* studies. This also fulfils most of the requirements of mineral loss or gain, in addition to its being precise and its ease of handling that is in agreement with other researchers who found using profilometry in the measurement of tooth surface loss offered a useful apparatus for quantifying the depth of enamel surface loss in *in vitro* and *in situ* studies (Eisenburger et al., 2000, Attin et al., 2001, Ganss et al., 2007a, Vieira et al., 2007, Magalhaes et al., 2008b). Abrasive wear resistance of acidic softened enamel increases with remineralising period and an interval of at least 1 hour was suggested before tooth brushing after an erosive attack (Attin et al., 2001).

6.9.2 Tooth brushing habits as a part of dental health education

Tooth brushing with fluoride containing toothpastes twice a day was considered and emphasised as a part of achieving dental health education to reduce enamel demineralisations by frequent consumption of demineralising drinks or food (Duggal et al., 2001). Also, it was found that in a cross-over in situ/ex vivo study with two phases of 7 days that was performed in 10 volunteers to assess the effect of fluoride dentifrice on eroded enamel by immersion of each appliance that held three human enamel specimens in a cola drink for 5 mins, 4 times a day and subjected to brushing abrasion with Crest dentifrice (pH 6.8; silica as abrasive; Procter and Gamble, Mason, Ohio, USA), or the control group without fluoride. Enamel alterations were measured using profilometry and percentage change in surface microhardness (%SMHC) and found that fluoride dentifrice had a protective effect on eroded enamel compared to that brushed with a non-fluoridated paste ($p=0.04$)(Magalhaes et al., 2007).

Tooth brushing timings after exposure to erosive substances are very important in reducing the resultant surface loss as demonstrated by erosive/ abrasive procedures

performed in 7 females in an *in situ* study that showed that the immersion of human enamel slabs in 20 ml citric acid solutions (0.1 M; pH = 3.5) for 3 mins under constant agitation at room temperature (20°C), then the eroded specimens were inserted into the lower appliances worn by 7 female subjects and brushed immediately (intra-oral exposure interval = 0 min) or worn for the experimental time period (30 and 60 mins). At the end of the intra-oral exposure intervals the test subjects had to brush the lower buccal sites for 30 s each using their preferred technique. A new toothbrush with 800 mg of Elmex® red toothpaste was used for each set of specimens. In that experiment, it was found that the 60 mins exposure to the oral environment was better than after 0 or 30 mins ($p<0.001$) (Jaeggi and Lussi, 1999).

However, in that *in situ* study the researchers did not take into account the repeated erosive and abrasive consequences over the extended timing period as compared to the present study, as tooth surface loss is considered to be a cumulative phenomenon over time (Zero and Lussi, 2000, Lussi et al., 2004b).

In another in situ study investigating the wear of eroded enamel with and without tooth brushing, the immersion time in the erosive solution was 10 mins, 4 times a day and the result demonstrated that the erosive wear without tooth brushing was less than that for erosion with tooth brushing (Rios et al., 2006). However 10 mins immersion could be an excessive erosive exposure duration and the frequency of 4 times daily does not follow the recommendations of 5 exposures a day.

Enamel dental wear was more pronounced when associated with tooth brushing abrasion. However, tooth brushing alone promoted less %SMHC due to the removal of the altered superficial enamel layer (Rios et al., 2006).

In the present study incorporation of two therapeutic anti-erosive agents was based on the conclusion of other studies that suggested the synergistic effects of erosion and tooth brushing abrasion that increased dental hard tissue wear and needed more appropriate therapy to minimise these synergistic effects (Jaeggi and Lussi, 1999, Rios et al., 2006).

The effect of different periods of intra-oral remineralisation on tooth brushing abrasion was studied on human enamel specimens which were eroded twice a day for 21 days extra-orally by exposure to a carbonated drink (Sprite light) twice a day extra-orally for 90 s. The specimens were then held for 0, 10, 20, 30 or 60 mins on intra-oral appliances worn by 8 subjects and then subsequently brushed extra-orally with an electric tooth brush and normal abrasive fluoride toothpaste. After 21 days, enamel wear was measured with a laser profilometer. Although the authors concluded that abrasion resistance of softened enamel increased with longer remineralisation periods, even after the intra-oral 60 mins remineralisation period, the wear significantly increased as compared to the demineralised but non-brushed controls. Analysis of variance revealed a significant influence of the remineralisation period on abrasive wear. However, even after a remineralisation period of 60 mins the wear significantly increased as compared to the demineralised, but non-brushed controls and it was observed that the tooth brushing abrasion resistance of softened enamel increased with the remineralisation period and it was suggested that a period of at least 60 min should pass before tooth brushing after an erosive exposure (Attin et al., 2001).

It is a common finding that the tooth wear of enamel and dentine is enhanced by the combination of soft drink erosion and toothpaste abrasion. Enamel itself appeared

resistant to abrasion compared to dentine and similarly more resistant to erosion than dentine. Therefore it was emphasised to avoid tooth brushing immediately following the consumption of acidic beverages (Hooper et al., 2003).

6.9.3 Wash-out period

To eliminate any carry-over effects of the previous test product a wash-out period of 2 days was suggested in between and prior to the start of each phase.

Salivary fluoride levels following application of fluoride varnish or fluoride rinse with NaF returned to baseline, on average, within 2 h for the NaF rinse and within 24 h for the varnish. The maximum fluoride levels were significantly greater (p<0.01) with the varnish than with the rinse and remained above baseline levels for a longer duration (Eakle et al., 2004). Salivary fluoride levels with NaF rinse returned to baseline, on average within 2 h while they remained elevated for, on average, 24 h with the varnish. Salivary fluoride levels from the varnish were found to be comparable with those in previous studies for 1.1% neutral NaF(Eakle et al., 2004). The two day lead-in and in-between the cross-over experimental wash-out period phases designed with the use of standard non-fluoride® toothpaste (Boots, UK) did not promote any significant risk of enamel loss that could be enhanced by using non-fluoridated toothpaste. In a crossover *in situ* study with 5 days of treatments (Ganss et al., 2007a) it was found that brushing without fluoride toothpaste increased the enamel surface loss significantly ($p \leq 0.001$). Furthermore, a 2 day wash-out period was thought to possibly optimise the participant's compliance during the study.

6.9.4 Use of fluoride mouthrinse

Using a fluoride rinse (226 ppm F) resulted in a higher increase of salivary fluoride levels than fluoride toothpastes applied by tooth brushing (Zero et al., 1992, Zero et al., 1988a). It was found that the level of fluoride and the method of delivery were very important as it had a major influence on F retention in the oral cavity. After 1-2 h of fluoride toothpaste treatment, plaque F values were only slightly higher than baseline values, while plaque F values were twice as high as baseline values for the fluoride rinse treatment. In addition to other influencing factors such as fluoride concentration and the time of applications (whether night or day time) for elevating the salivary fluoride in the mouth whereas, night-time fluoride application resulted in continued F preservation in whole saliva but not in plaque (Zero et al., 1992). The remaining amount of fluoride in the mouth after oral fluoride application, recovered during and immediately after application subtracted from the dose applied was calculated to be 10-14% of the original amount applied for the fluoride toothpaste and fluoride rinse treatments (0.17-0.24 mg F) and from 20-29% for the fluoride gel treatment (0.68-0.99 mg F) (Zero et al., 1992).

Samples were collected at baseline, immediately after treatment (0), then at 5, 15, 30, and 45 mins then at 1, 2, 6, 12, and 24 h after treatment.

Applying other methods for gaining maximum protection against tooth brushing abrasion and acidic erosive dental wear other than mouthrinses were studied in some in situ models by using e.g. high concentration fluoride to protect against tooth erosive wear such as fluoride varnish (Vieira et al., 2007). The protective layer of the fluoride varnish over the enamel samples were subjected to partial loss after brushing and erosive exposure with fluoride toothpaste leaving exposed enamel

surfaces which were detected visually and confirmed by optical profilometry. For that reason, the product should not be effective as a daily choice for protection against tooth surface wear as it needs repeated applications or should be smeared in multiple layers.

Literature review revealed in another 5 treatments cross-over in situ study combining two protocols to study the interplay between erosion and abrasion involving 15 volunteers and each volunteer wore an upper removable appliance that held one enamel and one dentine specimen from 09:00 am to 17:00 pm for 10 working days and only kept outside the mouth for 1 hour at lunch time. Furthermore no other foods or drinks except tea, coffee or water were permitted while the appliances were worn. During each study period, the subjects were randomly allocated to one of the five treatment regimens; (1) Drinking mineral water and brushing specimens with fluoride toothpaste A (RDA 189.074.0, REA 1. 2.070.7) ex vivo; (2) Drinking mineral water and brushing specimens with fluoride toothpaste B (RDA585.073.0, REA 3.470.3) ex vivo; (3) Drinking orange juice; (4) Drinking orange juice and brushing specimens with toothpaste A ex vivo; (5) Drinking orange juice and brushing specimens with toothpaste B ex vivo.

The erosive exposure was performed intra-orally by sipping 250 ml orange juice under supervision over 10 minutes at pre-determined periods at 09:00 am, 11:00 am, 13:00 pm and 15:00 pm leaving 1 hr. in between the erosion exposures and instantly after drinking, the appliances were removed from the mouth and rinsed under tap water. The brushing procedures of the specimens for 60 s were performed using one of the two commercially available fluoridated toothpastes with different abrasives and later on the specimens were replaced in the appliances and returned to the subjects. For the erosion only group with drinking orange juice, appliances were

kept in a moist condition during brushing of the other experimental groups. A wash-out period of at least two and a half days was allowed between each 10-day study period. Fresh enamel and dentine specimens were inserted in the appliances at the beginning of each study period. Finally, measurements of each specimen were taken using a profilometer at the end of days 5 and 10. However, the subjects were restricted from performing any oral hygiene procedures when the intra-oral appliances were worn and normal oral hygiene practices were allowed before or after each daily study period (0900 to 1700 h). This is in contradiction to the present study in which the subjects were asked to refrain from any other oral hygiene products except for the allocated test products and the brushing instructions were to brush one minute outside the mouth and brushing the teeth intra-orally. This was to stimulate natural saliva and toothpaste slurry for keeping the appliance and the attached enamel specimens for another 1 minute inside the mouth and the toothpaste was a specialised formula against erosion and also contained toothpaste alone or combined with an oral rinse. The results showed that orange juice was more highly erosive than water ($p<0.001$).

The erosive potential of seven oral care products was investigated on human enamel specimens that were distributed into seven groups of 10 specimens. The test solutions were liquefied where necessary, e.g. toothpastes were diluted with water (1/3 slurry)] and orange juice was a positive control. The enamel specimens were immersed for 10 and 20 minutes in the respective test solutions. Later on, the surface microhardness was measured for each slab and the results revealed that none of the tested oral products showed erosive loss in human enamel specimens. However, the tested oral products were investigated for their anti-erosive potential in comparison with orange juice for only 10 and 20 minutes. An in vivo study on 20

subjects to compare the effect of toothpastes containing SnF_2 (0.4% SnF2, 1.0% stannous pyrophosphate: 0.10% F–), or NaF (NaF, 0.15% F–) on enamel dissolution with diluted citric acid (100mmol l^{-1} or 10mmol l^{-1}) applied using a peristaltic pump (5 ml 7mlmin^{-1}). The acid was collected in a test tube before and after application of the respective toothpastes (etch I and etch II). On the labial surfaces of 4 healthy maxillary incisors (2 pairs of teeth for each patient (20 subjects) were included in the experiment). Then, four applications of the respective test toothpastes (SnF toothpaste and NaF_2 toothpaste) each for 1 min duration, then gently rinsed with water and finally the analysis was assessed by measuring the calcium content in the citric acid applied before and after the treatment with the test toothpaste. The results showed that the SnF_2 toothpaste markedly reduced the dissolution of teeth *in vivo* whereas, the NaF toothpaste delivered no protection (Young et al., 2006). It can be differentiated from the present *in situ* study in that the toothpaste application procedure on the teeth lasted for 4 mins and was divided into four separate 1 min applications, which was longer than the average time of a tooth brushing procedure (2 minutes). However, it can be disputed that the toothpaste application on the teeth in their experiment might be less effective than application by tooth brushing and toothpaste under normal daily life conditions, where normal tooth brushing allows greater delivery of the toothpaste to the tooth surface as it combines with the saliva in the oral cavity. On the other hand, the combination of toothpaste and physical movements of a toothbrush might result in mechanical mineral loss which should also be noted that the authors study only estimated the effect of one application (or four short applications) on the teeth. It is possible that daily use over a period of weeks or months might give an enhanced effect.

The present study results are in accordance with the results obtained by (Barlow et al., 2009), in their three clinical in situ studies that investigated the relative performance of commercially available and experimental dentifrice formulations. The first study (A) involved 58 subjects that used the following dentifrices: Sensodyne Pronamel (1450 ppm F as NaF/5% KNO_3); Blend-a-Med Classic (1450 ppm F as NaF); and a matched (Pronamel) placebo control (0 ppm F). The second study (B) involved 56 subjects with the following test dentifrices: Sensodyne Pronamel (1150 ppm F as NaF/5% KNO_3); Crest Cavity Protection (1100 ppm F as NaF); Crest Pro-Health (0.454% SnF_2 (1100 ppm F)/sodium hexametaphosphate); and a matched (Pronamel) placebo control (0 ppm F). The third study (C) involved 56 subjects with the following test dentifrices: Sensodyne Pronamel (1150 ppm F as NaF/5% KNO_3); Sensodyne Pronamel Gentle Whitening having different fluoride sources and remineralising agents.

The three *in situ* experiments were conducted using bovine enamel slabs that were previously softened using a dietary acidic challenge (grapefruit drink) for 25 minutes in vitro). Subjects wore their palatal appliances holding eight bovine enamel blocks, previously exposed for 25 minutes to an in vitro erosive challenge with grapefruit juice, for the duration of the experiment. Five minutes after appliance insertion, subjects undertook a supervised, 90-second brush/rinse regimen with their assigned dentifrice. Surface microhardness (SMH) of the specimens was determined prior to the erosive challenge (baseline). After the in vitro erosive challenge the enamel slabs were re-measured after four hours in situ remineralisation following the tooth brushing event. Finally, SMH values were also determined after a second in vitro erosive challenge after the in situ remineralisation. All three studies demonstrated a significantly greater percentage surface microhardness recovery (% SMHr) and

percentage relative erosion resistance for dentifrices containing sodium fluoride compared to placebo controls. Overall, a significantly greater % SMHr ($p<0.0001$) was observed for Sensodyne Pronamel compared to Blend-a-Med Classic, Crest Pro-Health, and Colgate Sensitive Multi Protection dentifrices. Similarly, Sensodyne Pronamel delivered a directionally better %RER (relative erosion resistance) vs. Blend-a-Med Classic ($p=0.0731$), and significantly higher %RER vs. Crest Pro-Health ($p=0.0074$) and Colgate Sensitive Multi Protection ($p<0.0001$). Crest Cavity Protection demonstrated a significantly better %RER ($p=0.031$) than Crest Pro-Health, which in turn demonstrated a significantly better %RER than the placebo control ($p<0.0001$). The results of these in situ studies support the effectiveness of dentifrices containing sodium fluoride to re-harden enamel previously softened with an erosive challenge. Furthermore, these studies demonstrated the protective effects conferred to enamel after erosive exposure following the remineralisation process in the presence of "ionic" fluoride. Under clinically relevant conditions, Sensodyne Pronamel and Sensodyne Pronamel Gentle Whitening offered superior anti-erosion performance compared to currently marketed dentifrice controls. These studies reinforce previous research indicating the importance of formulation effects on the relative remineralisation. Although the researchers *in situ* study involved a single tooth brushing and prolonged exposure to the erosive attack (25 minutes duration) and perhaps might not reflect the normal daily life style oral habits. Furthermore their studies did not measure the repeated alternating erosive and abrasive challenges as performed in the current *in situ* erosive and abrasive study.

A five treatment *in situ* study was performed in eight subjects to test the effect of fluoride rinsing on the prevention of tooth brush abrasion of softened enamel by immersion in 0.1 M citric acid (pH 3.5) for 3 mins. Four slabs per phase were

attached to intra-oral appliances and the treatments were as follows: (1) no softening, no fluoride rinsing (control 1); (2) softening, no fluoride rinsing (control 2); (3) softening, rinsing in situ with a sodium/amine fluoride rinsing solution (250 ppm F) for 30 s; (4) rinsing in situ with a sodium/amine fluoride rinsing solution (250 ppm F) for 30 s, softening; (5) softening, rinsing in situ with an experimental amine fluoride-containing rinsing solution (250 ppm F) for 30 s. The test subjects rinsed with 10 ml for 30 s, and, further, after an intra-oral exposure period of 1 h, the test subjects had to brush the lower buccal sites for 30 s, each using their preferred technique with a new toothbrush (Paro M 43®, Esro AG, Thalwil, Switzerland) with 800 mg of toothpaste (Elmex® red, Gaba AG, Therwil, Switzerland). Tooth brushing abrasion was measured on five series of enamel specimens on five consecutive days and on the sixth day saliva analysis was assessed. It was found that the loss of tooth substance was determined as toothbrush abrasion *in situ* which was not significantly lower using the fluoride rinsing solutions before or after softening the enamel compared to no rinsing ($p>0.05$). Multiple linear regression analyses revealed that 57% of the variation in tooth brush abrasion could be attributed to the severity of softening ($p<0.001$) and the pH of stimulated saliva ($p<0.001$). It was concluded that a single rinse for 30 s with a 250 ppm fluoride solution before or after acid exposure had no statistically significant effect on the prevention of tooth brush abrasion of softened enamel (Lussi et al., 2004a).

The *in situ* study of Lussi and co-workers(2004a) a lower effectiveness of the fluoride rinse on the prevention of tooth brush abrasion of softened enamel could probably be because of the very short rinsing time with the mouth rinse which was 30 s compared to the present study in which the rinsing time was 1 minute and twice per

day and followed tooth brushing with the assigned specialised toothpaste against erosion.

The retention of fluoride in the oral environment over 24 hours after administration of fluoride dentifrice or combined with mouth rinse was assessed in groups of 10 consenting adult subjects (age 18-52 years). The subjects brushed and/or rinsed (B/R) in a standardised manner twice per day in the morning (AM) and before bed (PM) with either a placebo dentifrice (8 ppm F), NaF dentifrice (1100 ppm F), or NaF rinse (225 ppm F). Experiments were performed with placebo dentifrice only (PD); F dentifrice only (FD); F dentifrice followed by F rinse (FD/FR); placebo dentifrice followed by F rinse (PD/FR); and F rinse followed by placebo dentifrice (FR/PD).

The investigators suggested that after measuring the salivary flow rate and for each of the sampling intervals unstimulated whole saliva samples which were collected at baseline and then at 0, 15, 30, and 45 mins, 1, 2, and 8 hr after B/R in the AM, after B/R in the PM and upon rising the following morning. F rinsing with fluoride rinse was shown to be more effective in delivering fluoride intra-orally than the fluoride dentifrice alone. However, based on F retention in the oral cavity the combination of fluoride dentifrice and mouth rinse was not more effective than a fluoride rinse alone or for the placebo plus fluoride rinse. Furthermore they suggested older individuals with gingival recession retained higher F levels; and bedtime fluoride application resulted in longer F retention than did daytime application, which may have important implications for enamel remineralisation (Zero et al., 1988b).

In the present study, all the test products were formulated against dental erosion except for the control non-fluoride toothpaste. Despite the repackaging and covering of the whole test products with white sheets, the taste sensitivity of these experimental products cannot be changed because each product has a different flavouring system. In addition to the other different added benefits, the ingredients present in the modern oral care health products which may affect the subject's personal taste preferences for the assigned products.

6.10 Conclusions

1. This *in situ* study seemed to be a good model for investigating combined tooth brushing abrasion and acidic erosive challenge using a combination of products.

2. The use of small concentrations of fluoride in the mouth rinses which is designed to protect against erosion is effective in conjunction with the daily use of special formulations of toothpastes against erosion.

3. The treatment with Elmex® erosion protection toothpaste and Elmex® erosion protection rinse had a considerable effect in reducing the erosive and abrasive enamel wear compared to Elmex® erosion protection toothpaste alone or Sensodyne® Pronamel toothpaste alone $p \leq 0.006$ and $p \leq 0.001$ respectively.

4. Toothpastes (Sensodyne® Pronamel and Elemex® erosion protection) designed to prevent dental erosion showed a protective effect in erosive/ abrasive dental tissue loss compared to non–fluoride® toothpaste ($p \leq .001$).

5. Although there was no statistical significant differences observed between Sensodyne Pronamel® toothpaste plus mouth rinse and Elmex® erosion protection toothpaste and rinse, Elmex® erosion protection toothpaste and rinse, showed a slightly better efficacy over Sensodyne Pronamel® toothpaste plus mouth rinse as demonstrated in the earlier *in vitro* study of the present group of studies (two *in vitro* and one *in situ* studies).

6. The treatment by therapeutic products Elmex® erosion protection toothpaste, Sensodyne Pronamel® toothpaste, Sensodyne Pronamel® toothpaste plus mouth rinse and Elmex® erosion protection toothpaste and rinse demonstrated a statistical significant reduction in the erosive and abrasive enamel surface loss (µm) compared to treatment with non-fluoride® toothpaste ($p \leq 0.001$).

7. There were no statistical significance between Elmex® erosion protection toothpaste and Sensodyne Pronamel® toothpaste ($p>0.05$).

8. There were no statistical significance between Sensodyne Pronamel® toothpaste plus Sensodyne Pronamel® mouth rinse and Sensodyne Pronamel® toothpaste ($p\leq0.1$) or Elmex® erosion protection toothpaste ($p=0.250$) or Elmex® erosion protection toothpaste and Elmex® erosion protection rinse ($p=0.106$).

The general conclusions of the entire studies in this research thesis are that not all fluoridated toothpastes have similar preventive effects on enamel surface loss. The following therapeutic products such as Elmex *anti-caries*, Elmex Sensitive Plus, Elmex erosion protection and Sensodyne Pronamel had better protective effects against erosion compared with Meridol and non-fluoride Aronal.

The therapeutic combinations Elmex erosion protection toothpaste and Elmex erosion protection mouth rinse, Sensodyne Pronamel in combination with Sensodyne mouth rinse had better protective effects than toothpastes alone.

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8 APPENDICES

8.1 Appendix 1: KHS baseline measurements of bovine enamel

Table 8.1 Knoop microhardness measurements of bovine enamel slabs

Number of slab	1st reading	2nd reading	3rd reading	The average
B11	65.6	61.9	60.1	62.5
B12	66	65.9	68.2	66.7
B13	66.2	67.3	66.9	66.8
B14	67.5	66.7	63.7	66.0
B15	66	65.5	67.9	66.5
B16	62.7	65.8	64.7	64.4
B17	60.7	65.8	67.1	64.5
B18	60.8	65.8	64.7	63.8
B19	61.8	61.7	63.4	62.3
B20	61.7	64.9	66.7	64.4
B21	64.9	66.8	65.8	65.8
B22	61.3	62.9	60.8	61.7
B23	60.3	64.3	62.9	62.5
B24	67.1	65.9	66	66.3
B25	68.1	64.8	66.8	66.6
B26	61.9	63.9	64.7	63.5
B27	65.8	67.8	65.4	66.3
B28	66.4	62.3	61.4	63.4
B29	66.7	65.1	64.8	65.5
B30	67.1	63.8	67.3	66.1
B31	63.4	65.1	64.9	64.5
B32	66.9	67.1	65.9	66.6
B33	66.4	65.7	63.9	65.3
B34	63	65.3	67.2	65.2
B35	61.9	65.2	63.9	63.7
B36	63.2	67.3	65.4	65.3
B37	61.2	60.8	62.5	61.5
B38	65.9	66.5	64.9	65.8
B39	64.5	66.2	65.9	65.5
B40	62.5	60.8	61.2	61.5
B41	65.2	64.3	66.1	65.2
B42	63	61.9	60.9	61.9
B43	67.2	65.9	66.6	66.6
B44	68.1	66.8	65.7	66.9
B45	67.8	66	64.9	66.2
B46	63.2	60.1	65.4	62.9
B47	64.8	65.2	63.9	64.6
B48	66.7	65.9	67.4	66.7
B49	67.8	66.4	62.6	65.6
B50	64.8	65.9	67.4	66.0

8.2 Appendix 2: KHS baseline measurements of human enamel

Table 8.2. Knoop microhardness measurements of human enamel

Number of slab	1st reading	2nd reading	3rd reading	The average
H11	61.7	66.4	62.7	63.6
H12	60.8	60.3	59.9	61.7
H13	61.9	60.1	61.2	61.1
H14	61.7	59.7	59.5	60.3
H15	61	59.7	60	60.2
H16	64.8	64.1	61.7	63.5
H17	64.3	60.9	65.5	63.6
H18	59.8	60.1	62	60.6
H19	59.9	60.3	59.7	60.0
H20	61.9	62.7	59	61.2
H21	59	63.2	59.5	60.6
H22	60	59.9	60.6	60.2
H23	63	60.3	59.7	61.0
H24	60.1	59	60.8	60.0
H25	61.2	61.2	61.4	61.3
H26	59.7	59.7	60.8	60.1
H27	60.6	60.3	59	60.0
H28	60	59	61	60.0
H29	59.5	60.1	60.2	59.9
H30	60.6	60.3	59	60.0
H31	59.8	62.3	63.4	61.8
H32	59.9	63.1	63.5	62.2
H33	65.1	64.5	63.8	64.5
H34	63	62.3	63.7	63.0
H35	64.1	64.3	61.9	63.4
H36	65.1	64.4	63.8	64.4
H37	64.1	64.3	61	63.1
H38	59.3	60.1	61.4	60.3
H39	60.9	59.3	60	60.1
H40	60.9	59.9	62	60.9
H41	59.9	61.3	60.1	60.4
H42	63	61.9	60.9	61.9
H43	65	62.5	65.9	64.5
H44	61.7	61.4	61.2	61.4
H45	61.9	62.7	66.1	63.6
H46	62.7	61.9	61.2	61.9
H47	60.1	62.5	63.6	62.1
H48	62.7	60.8	60.1	61.2
H49	64.4	63.8	65.2	64.5
H50	60.9	59.8	61.9	60.9

8.3 Appendix 3: The toothbrush that was used for the *in vitro* mechanical brushing

Figure 8.1 toothbrush used for *in vitro* mechanical brushing

8.4 Appendix 4: The Profilometry Scanning for bovine enamel

(a) Meridol group (E1)

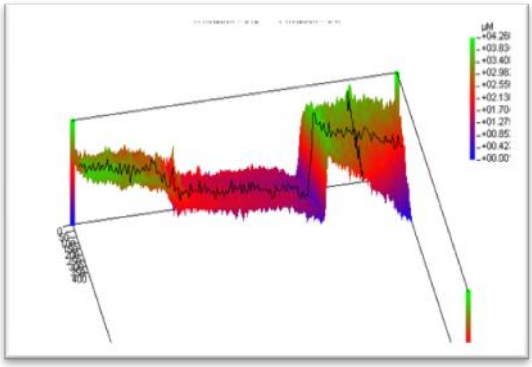

Figure 8.2 Image of enamel slab treated with Meridol at the initial experimental cycling period.

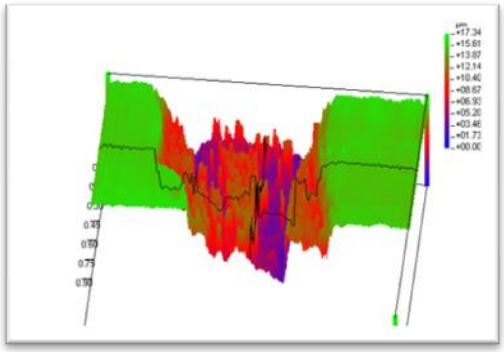

Figure 8.3 Image of enamel slab treated with Meridol at the end of experimental cycling period.

(b) Images Elmex group(E2)

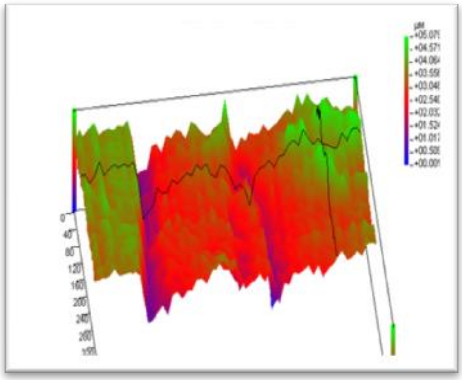

Figure 8.4 Image of enamel slab treated with E2 at the initial experimental cycling period.

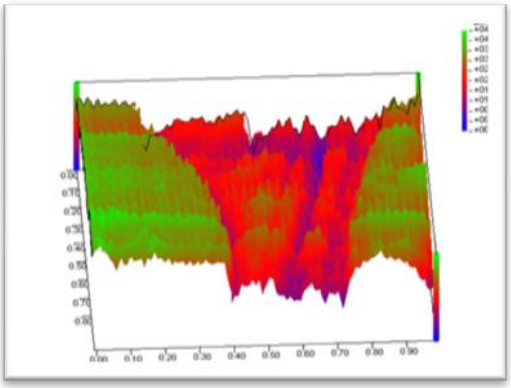

Figure 8.5 Image of enamel slab treated with E2 at the end of experimental cycling period.

(c) Pronamel group (E3)

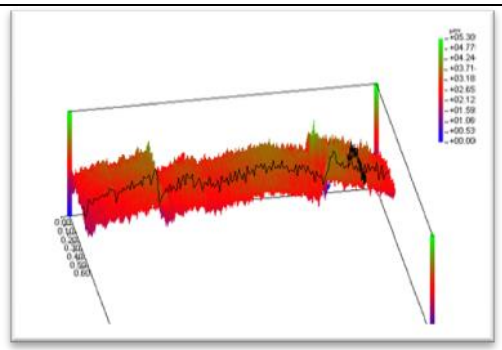

Figure 8.6 Image of E3 during initial period of cycling.

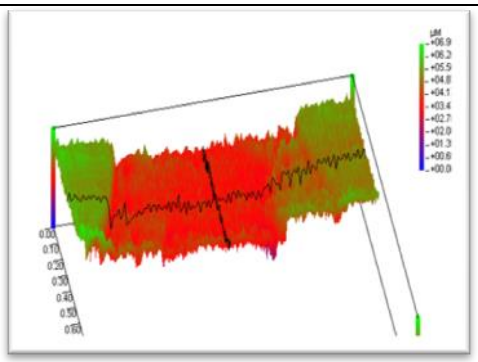

Figure 8.7 Image of E3 at the end of cycling period.

(d)Elmex sensitive (E4)

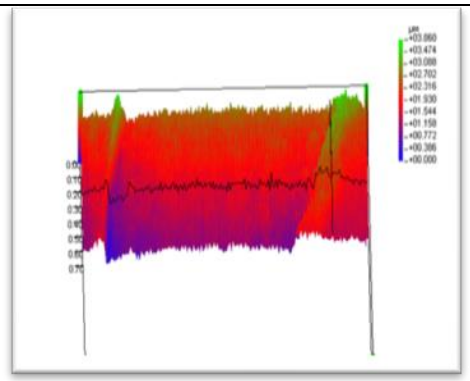

Figure 8.8 Image of E4 during initial period of cycling.

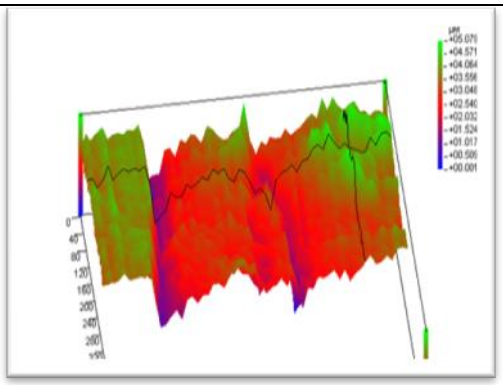

Figure 8.9 Image of E4 during the end of cycling period.

(e)Control groups (E5)

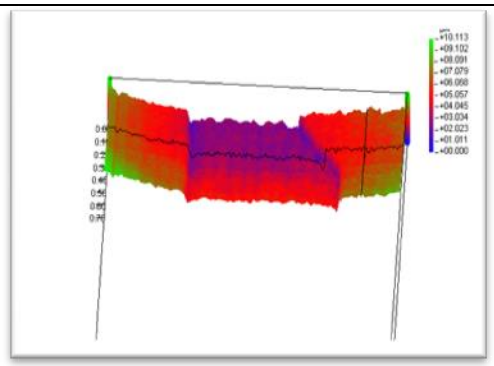

Figure 8.10 Image of E5 during initial period of cycling.

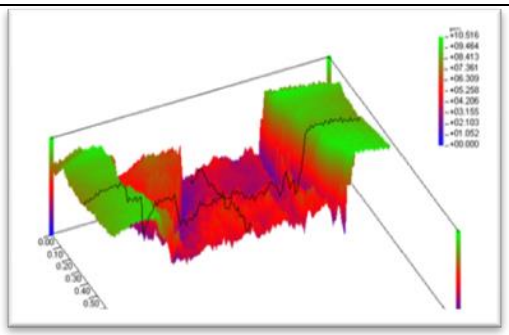

Figure 8.11 Image of E5 during the end of cycling period.

8.5 Appendix 5: The Kolmogorov-Smirnov tests for all experimental groups for bovine enamel

Normal distribution of each group during the 4 phases of the experimental periods of bovine ESL procedures was checked by using non-parametric tests as shown in Tables (8.3-8.4, 8.5, 8.6) showed that 2-tailed p>0.05 indicated a normal distribution of all data.

Table 8.3 One sample Kolmogorov-Smirnov test after 7 days

For bovine after7days		**E1**	**E2**	**E3**	**E4**	**E5**
N (Number of slabs)		9	10	10	10	10
Normal Parameters(a,b)	Mean	5.29	0.697	0.96	1.03	3.36
	SD	7.71	0.77	1.31	0.83	5.15
Most Extreme Differences	Absolute	0.25	0.24	0.33	0.16	0.36
	Positive	0.25	0.24	0.33	0.155	0.36
	Negative	-0.25	-0.20	-0.23	0.130	0.28
Kolmogorov-Smirnov Z		0.76	0.75	1.05	0.49	1.13
Asymp. Sig. (2-tailed)		0.61	0.62	0.22	0.97	0.15

a. Test distribution is Normal.
b.Calculated from data.

Table 8.4 One sample Kolmogorov-Smirnov test after14 days

For bovine enamel after 14 days		**E1**	**E2**	**E3**	**E4**	**E5**
Number of slabs		**9**	**10**	**10**	**10**	**10**
Normal Parameters(a,b)	**Mean**	**6.07**	**1.72**	**1.17**	**1.80**	**2.47**
	SD	**4.97**	**1.66**	**.99**	**1.20**	**2.26**
Most Extreme Differences	**Absolute**	**.308**	**.306**	**.220**	**.158**	**.202**
	Positive	**.308**	**.306**	**.167**	**.158**	**.202**
	Negative	**-.183**	**-.167**	**-.220**	**-.157**	**-.200**
Kolmogorov-Smirnov Z		**.925**	**.969**	**.696**	**.501**	**.639**
Asymp. Sig. (2-tailed)		**.360**	**.305**	**.718**	**.963**	**.809**

Table 8.5 One sample Kolmogorov-Smirnov test after 21 days

For bovine enamel after 21 days		E1	E2	E3	E4	E5
N		9	10	10	10	10
Normal Parameters(a,b)	Mean	6.25	2.14	3.90	2.25	5.35
	SD	2.24	1.73	3.27	1.19	4.07
Most Extreme Differences	Absolute	.246	.122	.206	.163	.252
	Positive	.246	.122	.206	.163	.252
	Negative	-.146	-.111	-.139	-.144	-.129
Kolmogorov-Smirnov Z		.739	.386	.651	.515	.798
Asymp. Sig. (2-tailed)		.646	.998	.791	.954	.548

Table 8.6 One sample Kolmogorov-smirnov test after 28 days for bovine enamel

For bovine enamel after 28 days		E1	E2	E3	E4	E5
N		9	10	10	10	10
Normal Parameters(a,b)	Mean	8.89	2.09	2.96	1.95	6.13
	SD	2.94	1.125	1.72	1.01	3.03
Most Extreme Differences	Absolute	.195	.171	.203	.130	.285
	Positive	.171	.134	.203	.112	.285
	Negative	-.195	-.171	-.164	-.130	-.144
Kolmogorov-Smirnov Z		.585	.541	.643	.412	.900
Asymp. Sig. (2-tailed)		.884	.932	.803	.996	.393

8.6 Appendix 6 ANOVA results for bovine enamel

Table 8.7ANOVA test results bovine enamel for after 7 days

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	150.839	4	37.710	2.237	0.080
Within Groups	741.757	44	16.858		
Total	892.595	48			

There were no significant differences among the groups during 7 days cycling procedure (P=0.08). These groups considered no change in mean surface loss so these groups were considered baseline.

Table 8.8 ANOVA results for bovine enamel after 14 days

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	143.177	4	35.794	5.413	0.001
Within Groups	290.946	44	6.612		
Total	434.123	48			

P≤ 0.001 is highly significant and pairwise comparisons were performed

Table 8.9 ANOVA results for bovine enamel after 21 days

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	128.907	4	32.227	4.366	0.005
Within Groups	324.796	44	7.382		
Total	453.703	48			

Normally distributed and significant test

P≤.005

Table 8.10 ANOVA results for bovine enamel after 28 days

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	345.645	4	86.411	19.093	0.000
Within Groups	199.140	44	4.526		
Total	544.785	48			

ANOVA p value is highly significant

p≤ 0.001

8.7 Appendix 7: Some examples of profilometry scanning for human enamel

(a) Meridol group (E1)

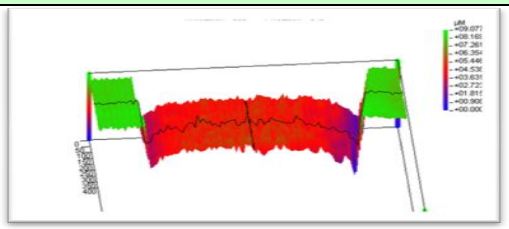

Figure 8.12 Image of E1 during the initial period for human enamel

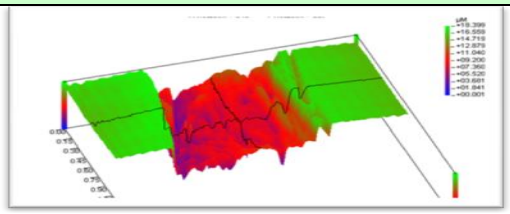

Figure 8.13 Image of E3 during the end of cycling periodfor human enamel

(a) Elmex group (E2)

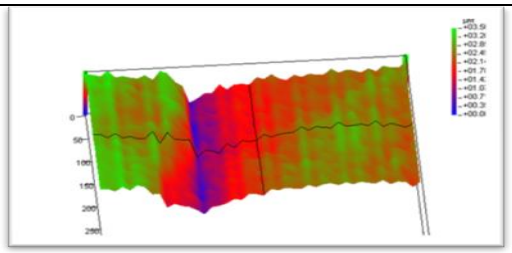

Figure 8.14 Image of E2 during initial period of cycling for human enamel

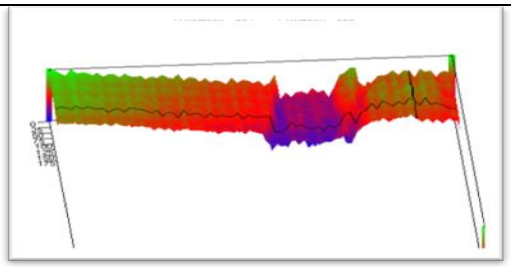

figure 8.15 Image of E2 during the end of cycling for human enamel

(c) Pronamel group (E3)

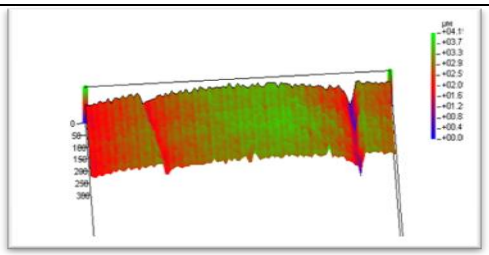

Figure 8.16 Image of E3 during the initial period for human enamel

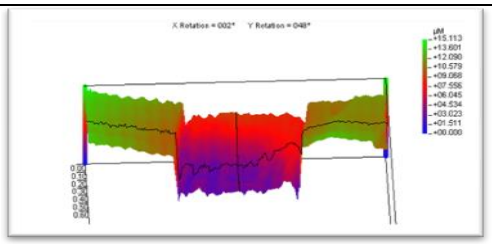

Figure8.17 Image of E3 at the end of cycling for human enamel

(b) Elmex sensitive (E4)

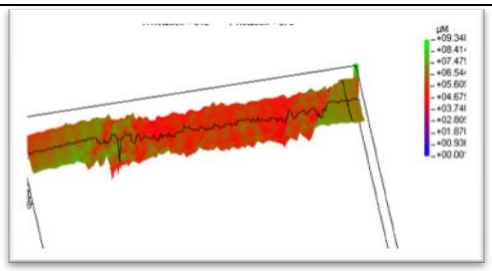

Figure 8.18 Image of E4 during the initial period of cycling in human enamel

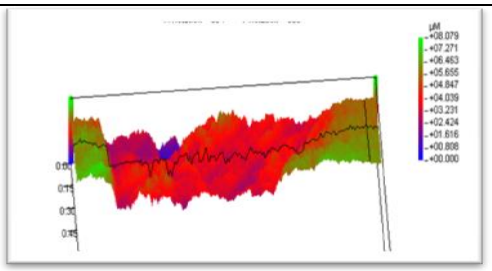

Figure 8.19 Image of E4 at the end of cycling in human enamel

(c) **Aronal, Control group) (E5)**

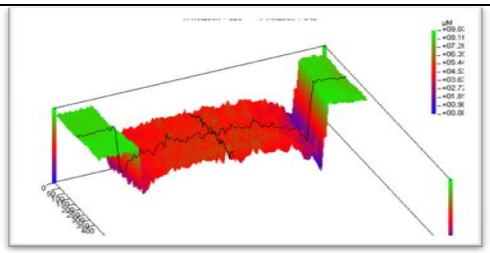

Figure 8.20 Image of E5 during initial cycling in human enamel

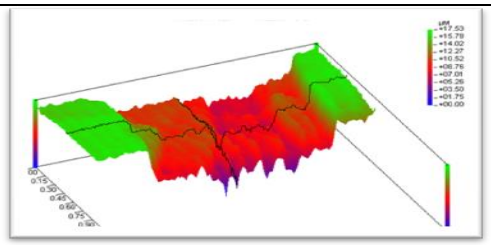

Figure 8.21 Image of E5 at the end of cycling in human enamel

8.8 Appendix 8: Kolmogorov-Smirov tests for human enamel

NPar Tests

Table 8.11 Kolmogrov-Smirov tests for human enamel after 7 days

		E1	E2	E3	E4	E5
N		10	10	10	10	10
Normal Parameters(a,b)	Mean	3.07	1.32	1.38	1.11	3.17
	SD	2.53	.63	2.02	.776	1.57
Most Extreme Differences	Absolute	.283	.140	.373	.157	.199
	Positive	.283	.073	.373	.157	.121
	Negative	-.157	-.140	-.265	-.129	-.199
Kolmogorov-Smirnov Z		.895	.442	1.18	.498	.630
Asymp. Sig. (2-tailed)		.400	.990	.123	.965	.823

a Test distribution is Normal.

b Calculated from data.

Table 8.12 Kolmogorov-Smirnov tests for human enamel after 14 days

		grp1	grp2	grp3	grp4	grp5
N		10	10	10	10	10
Normal Parameters(a,b)	Mean	3.18	1.28	1.39	1.78	2.92
	SD	2.19	.76	1.64	.89	1.49
Most Extreme Differences	Absolute	.295	.244	.287	.155	.183
	Positive	.295	.244	.287	.123	.109
	Negative	-.180	-.137	-.212	-.155	-.183
Kolmogorov-Smirnov Z		.932	.771	.908	.491	.580
Asymp. Sig. (2-tailed)		.350	.592	.381	.970	.890

a Test distribution is Normal.

b Calculated from data.

Table8.13 Kolmogorov-Smirnov tests for human enamel after 21 days

One-Sample Kolmogorov-Smirnov Test		grp1	grp2	grp3	grp4	grp5
N		10	10	10	10	10
Normal Parameters(a,b)	Mean	5.34	1.99	3.10	2.35	5.54
	SD	4.32	.68	2.24	.84	3.04
Most Extreme Differences	Absolute	.310	.136	.332	.150	.184
	Positive	.310	.136	.332	.114	.184
	Negative	-.244	-.092	-.217	-.150	-.140
Kolmogorov-Smirnov Z		.982	.429	1.05	.473	.583
Asymp. Sig. (2-tailed)		.290	.993	.222	.979	.886

a Test distribution is Normal.

b Calculated from data.

Table 8.14 Kolmogrov-Smirnov tests for human enamel after 28 days

One-Sample Kolmogorov-Smirnov Test		E1	E2	E3	E4	E5
N		10	10	10	10	10
Normal Parameters(a,b)	Mean	9.03	2.43	3.31	2.96	6.11
	SD	7.47	1.16	2.519	1.550	2.39
Most Extreme Differences	Absolute	.318	.306	.247	.210	.242
	Positive	.318	.306	.247	.210	.242
	Negative	-.255	-.231	-.190	-.140	-.116
Kolmogorov-Smirnov Z		1.01	.969	.780	.664	.765
Asymp. Sig. (2-tailed)		.263	.305	.577	.771	.602

a Test distribution is Normal.

b Calculated from data.

8.9 Appendix 9 ANOVA results for human enamel after 7, 14, 21, 28 days during erosion and abrasion cycling

Table 8.15 ANOVA results for human enamel after 7 days

1st wk HESL	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	41.250	4	10.313	3.699	.011
Within Groups	125.463	45	2.788		
Total	166.713	49			

Table 8.16 ANOVA results for human enamel after 14 days

2nd wk HESL	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	31.114	4	7.779	3.495	.014
Within Groups	100.165	45	2.226		
Total	131.279	49			

Table 8.17 ANOVA results for human enamel after 21 days

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	102.657	4	25.664	3.626	.012
Within Groups	318.517	45	7.078		

Table 8.18 ANOVA results for human enamel after 28 days

4th wk HESL	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	306.644	4	76.661	5.347	.001
Within Groups	645.179	45	14.337		
Total	951.823	49			

8.10 Appendix 10 Ethical approval

NHS
Health Research Authority

NRES Committee Yorkshire & The Humber - Leeds East
Yorkshire and Humber REC Office
First Floor, Millside
Mill Pond Lane
Meanwood
Leeds
LS6 4RA

Tel: 0113 30 50108
Fax: 0113 85 56191

05 April 2012

Dr Fowziya Ali
Paediatric Dentistry department,
Worsley Building, Level 6,
Clarendon Way, Leeds Dental Institute.
LS2 9LU

Dear Dr Ali

Study title:	**Investigations of therapeutic products on prevention of enamel surface loss under erosive and abrasive challenges**
REC reference:	**11/YH/0367**
Protocol number:	**NA**
Amendment number:	**1**
Amendment date:	**22 March 2012**

The above amendment was reviewed by Sub-Committee on 05 April 2012 in correspondence.

Ethical opinion

The members of the Committee taking part in the review gave a favourable ethical opinion of the amendment on the basis described in the notice of amendment form and supporting documentation.

Approved documents

The documents reviewed and approved at the meeting were:

Document	Version	Date	
Participant Information Sheet: Volunteer Information Sheet	2	08 February 2012	
Protocol	2	08 February 2012	
Notice of Substantial Amendment (non-CTIMPs)	1	29 February 2012	
Covering Letter		20 March 2012	

Membership of the Committee

8.11 Appendix 11 Ethics 2

The Leeds Teaching Hospitals NHS
NHS Trust

Ref: Amanda Burd

25/10/2011

Professor Monty Duggal
Paediatric Dentistry
Worsley Building,
Level 6, Clarendon Way,
Leeds Dental Institute
LS2 9LU

Research & Development
Leeds Teaching Hospitals NHS Trust
34 Hyde Terrace
Leeds
LS2 9LN

Tel: 0113 392 2878
Fax: 0113 392 6397

r&d@leedsth.nhs.uk
www.leedsth.nhs.uk

Dear Prof Monty Duggal

Re: NHS Permission at LTHT for: Investigations of theraputic products on prevention of enamel surface loss under erosive and abrasive challenges
LTHT R&D Number: DT11/10039
REC: 11/YH/0367

I confirm that *NHS Permission for research* has been granted for this project at The Leeds Teaching Hospitals NHS Trust (LTHT). NHS Permission is granted based on the information provided in the documents listed below. All amendments (including changes to the research team) must be submitted in accordance with guidance in IRAS. Any change to the status of the project must be notified to the R&D department.

Permission is granted on the understanding that the study is conducted in accordance with the *Research Governance Framework for Health and Social Care*, ICH GCP (if applicable) and NHS Trust policies and procedures available at http://www.leedsth.nhs.uk/sites/research_and_development/.

This permission is granted only on the understanding that you comply with the requirements of the *Framework* as listed in the attached sheet "Conditions of Approval".

If you have any queries about this approval please do not hesitate to contact the R&D Department on telephone 0113 392 2878.

Indemnity Arrangements

8.12 Appendix 12 Volunteer information sheet

Study Title: Investigations of therapeutic products on prevention of enamel surface loss under erosive and abrasive challenges

Introduction: You are being invited to take part in the above research study. Before you decide, it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully,

What is the purpose of the study?

The aim of our study is to see what products used for your routine oral hygiene work best to protect the enamel of teeth against acidic challenge.

What is the procedure that is being tested?

In our study we will use 5 different toothpastes and 2 mouth washes, either alone or in combination. All products are currently in use and commonly available in the supermarkets.

Why have I been chosen?

All we ask of volunteers is that they are willing to take part in the study; over18 years old; in general good health; that they are not pregnant or planning to become pregnant during the course of the study; having at least 18 natural teeth. Before you are enrolled on the study, you will need to be 'screened'. This will involve a short dental examination, to enable us to establish whether you meet all our criteria.

Do I have to take part?

Participation in this study is entirely voluntary. If you decide to take part, then you have to sign a consent form after clear explanation of the aims, objectives and methods of this study. Even if you decide you would like to take part in the study, but you later decide you no longer wish to continue, you can withdraw at any time,

What will happen to me if I take part?

The study consists of 5 parts with14 days each, therefore the full study time will be 3-4 months. You will be required to wear a removable mouth appliance which contains two sterilised enamel sections between 9am in the morning and 5pm in the evening and store it overnight in moistened conditions (it should be stored in moist tissue in a special container when removed from the mouth). These enamel sections will be from cow's teeth after being adequately sterilised. You need to visit us 3-4 times in each period, about once each week. These visits are to supply you with the device; solutions; and to collect it again at the end of that period. **What do I have to do?**

For each part of the study (lasting 14 days each), you will need to agree to wear the device we will construct for you from 9am to 5pm and to remove the device at specified times (during eating, drinking and overnight) and to dip the device in a citric acid solution five times per day in a special container provided. You will also need to brush and rinse the device using the special toothpaste and toothbrush provided to you two times per day and agree to come into the dental centre at certain dates and times. For two days between each part of the study, you will be asked to use only the toothbrush and toothpaste that has been provided to you. You will be asked to abstain from flossing, using oral and breath fresheners and using chewing gum. We realise that this may cause you a small amount of inconvenience at first, but once you get used to the routine, we feel you will find it easy to stick to.

What are the side effects of taking part?

We are very confident that no significant damage will be caused to you or your teeth if you take part in this study. The only slight risk could be a very small chance of enamel mineral loss, because you will be using fluoride-free toothpaste during the study. However, previous tests have shown that teeth regain lost minerals naturally by saliva and its use will be limited only for 2 days between each part of the study.

What are the possible disadvantages or risks of taking part?

The only disadvantage to you as a volunteer that you will be asked to wear and remove the device at the times specified. You may find this to be a slight inconvenience. However, we are sure you will find it easy to remember what to do and when.**What are the possible benefits of taking part?**

The information gained from the study may provide a better knowledge on prevention of tooth mineral loss. In recognition of any inconvenience and out of pocket expenses you will incur, you will be paid a fee of £150 for taking part in at the end of the

study. You will need to complete a bank details form, and provide us with your National Insurance Number. This information will be held confidentially.

What if new information becomes available?

If any new information becomes available we will of course let you know.

What happens when the research study stops?

At the end of the study, you will need to come back to the test centre. A dentist will give you a final check-up, and you will have a fluoride gel applied to your teeth.

What if something goes wrong?

If you have any concerns about your treatment during the study, please do not hesitate to contact any one of us, and we will do our best to help.

Will my taking part in this study be kept confidential?

Any information we gather will be kept confidential, your medical/dental records may need to be accessed in certain instances. You will not be identified by name in any reports or publications.

What will happen to the results of the research study?

The results from this study will be made public through publication in academic journals and at scientific meetings.

Who is organizing and funding the research?

The study will be carried out by the research team at the Leeds Dental Institute/University of Leeds. More specifically, the principal investigator is Dr Fowzia Ali and supervised by Head of Paediatric Dentistry /Prof. Monty Duggal, and consultant / Prof. Jack Toumba.

Contact for further information:

Dr Fowziya Ali (0113) 343 6138 dnfa@leeds.ac.uk

Professor Monty Duggal (0113) 343 6177 m.s.duggal@leeds.ac.uk

Out of hours contact No. 07728040793

Paediatric Dentistry, Leeds Dental Institute,

Clarendon Way, Leeds, LS2 9LU, UK

8.13 Appendix 13 Informed Consent Form

Title of Research Project: Investigations of therapeutic products on prevention of enamel surface loss under erosive and abrasive challenges

Name of Researcher: Fowziya Ali

I confirm that I have read and understand the information sheet dated *[insert date]* explaining the above research project and I have had the opportunity to ask questions about the project.

I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason and without there being any negative consequences. In addition, should I not wish to answer any particular question or questions, I am free to decline. *Insert contact number here of lead researcher/member of research team (as appropriate).*

I understand that my responses will be kept strictly confidential I give permission for members of the research team to have access to my anonymised responses. I understand that my name will not be linked with the research materials and I will not be identified or identifiable in the report or reports that result from the research

I agree for the data collected from me to be used in future research

I agree to take part in the above research project and will inform the principal investigator should my contact details change.

Name of participant	Date	Signature
(*or legal representative*)		
Fowziya Ali	_____	_____
Name of person taking consent	Date	Signature
(*if different from lead researcher*)		

To be signed and dated in presence of the participant

Fowziya Ali	_____	_____
Lead researcher	Date	Signature

To be signed and dated in presence of the participant

8.14 Appendix 14 Randomisation table

Distribution of the randomisation table (subject code numbers, 007, 015 and 017 were withdrawn from the study at an early stage)

Table 8.19 Distribution of the randomisation table for *in situ* study

Subject code number		Randomisation				
	Randomisation number	Phase 1	Phase 2	Phase 3	Phase 4	Phase 5
001	R 1	A+E	C	C+D	B	A
002	R 10	B	A	A+E	C	C+D
003	R 7	C+D	A+E	A	C	B
004	R 2	C+D	B	AE	C	A
005	R 3	C	A	A+E	B	C+D
006	R 4	C+D	B	C	A	A+E
007	R 3	C+D	A+E	A	C	B
008	R 5	A+E	A	C+D	C	B
009	R 14	C+D	A+E	B	A	C
010	R 16	B	C	A	C+D	A+E
011	R 12	C+D	C	A+E	A	B
012	R 15	B	C	A+E	A	C+D
013	R 11	A	B	A+E	C	C+D
014	R 9	A	B	C+D	C	A+E
015	R 12	B	C	A+E	A	C+D
016	R 14	B	A+E	C+D	A	C
017	R17	C+D	B	A	A+E	C
018	R 18	C	C+D	A+E	B	A
019	R 19	C	B	A	A+E	C+D
020	R 20	C+D	A+E	A	B	C

8.15 Appendix 15 Instruction sheet

Investigations of therapeutic products on prevention of enamel surface loss under erosive and abrasive challenges

INSTRUCTION SHEET

Study Investigator/Dentist11/YH/0367

Dr Fowziya Ali

Principal Investigators

Professor M. S. Duggal

Professor K. J. Toumba

Department of Paediatric Dentistry, Leeds Dental Institute

BRIEF INFORMATION FOR COMPLETION OF THE FOLLOWING DIARY INSTRUCTIONS

Wearing your device

- The device must be worn continuously from 9.00 am till 5.00 pm daily, except at mealtimes, whilst drinking, or during the night. Please remove your device when you are eating, drinking and overnight.

TEST PERIODS

Brushing your device:(During the experimental day)

- A test new tooth brush will be supplied to you at the beginning of each phase which we ask you to use.
- Dipping (brushing) and rinsing of the device with the provided test toothpaste and toothbrush should occur twice / a day in the morning and evening as described below:
- Dipping of the device into toothpaste will be achieved by brushing for 1 minute using the toothpaste supplied as normal and while the device is outside the mouth. Then the device will be re-inserted in the mouth and swashed using the toothpaste in the mouth for another 1 minute.
- If you are supplied with mouth rinse, please use the mouth rinse for rinsing after swishing (with the toothpaste) for a further 1min.
- If you are not supplied with a mouth rinse, please rinse with tap water after swishing (with the toothpaste) for a further 1min.

Citric acid dipping

- The citric acid is a natural product of most citrous fruits and usually used in commercial juices.

- Citric acid crystals will be supplied in plastic bottles. The weight of citric acid in each bottle will be enough for one day. Another plastic bottle will be supplied to mix the water with citric acid crystals. Please pour water into the plastic bottle so that it is level with the indication line marked on the bottle.

8.16 Appendix 16 Dipping diary log

Subject ID:	Randomisation no.:	Phase no.	1 June 2012

Study Investigator/Dentist
Dr Fowziya Ali
Dept of Paediatric Dentistry
Division of Child Dental Health, LDI

Principal Investigators
Professor M. S. Duggal
Professor K. J. Toumba
Dept of Paediatric Dentistry
Division of Child Dental Health, LDI

Contact details for further advice if a problem occurs with the appliance.

Researcher:

Dr Fowziya Ali

24 hour contact number Mobile: 07728040793

Research Coordinator:

Mrs. Gillian Dukanovic

Day time tel: 0113 3436127

Dipping Diary Sheet (14 days)

Test toothpaste +/_ mouthwash's start date. __ __ / __ __ / __ __ __ __

Dental device using start date. __ __ / __ __ / __ __ __ __

Dental device dipping start date. __ __ / __ __ / __ __ __ __

Date	Morning Brushing** for 1 minute then swishing for 1 minute & rinsing with tap water or swishing for 1 minute with mouth wash (if provided) * record the duration and at what time of day it was done	Dipping in citric acid solution***					Evening Brushing** for 1 minute then swishing for 1 minute & rinsing with tap water or swishing for 1 minute with mouth wash (if provided) * record the duration and at what time of day it was done	Please provide details if you had to leave the device out of your mouth for more than 1 hour or if you did not wear the device from 9am to 5pm
		1st time 10am 2min	2nd time 11am 2min	3rd time 12pm 2min	4th time 1pm 2min	5th time 2pm 2min		

*** Please record the exact duration of dipping and the time it takes place.**

**** Brush with the provided toothbrush/toothpaste for 1 minute with the device out of your mouth followed by 1 minute swishing in toothpaste whilst the device in the mouth. Rinse the device in your mouth with either tap water or test mouthwash if you have been provided with one, for a further 1 minute.**

***** Dip in citric acid for 2 minutes at each time point indicated and tick the boxes when this has been done if done at the times indicated. If the time is different, please record the time done.**

8.17 Appendix 17 Visit 1: Screening visit (page 1 of 2)

	YES	NO	STAFF INITIALS	COMMENTS (eg if not done)
Subject given copy of PIS (must have received PIS at least 3 days prior to first study visit)?	☐	☐		Write date PIS sent to subject
Has subject read PIS?	☐	☐		
Has subject refrained from any oral hygiene for 48 hours prior to visit (eg flossing, use of mouth and breath fresheners/mouthwashes)?	☐	☐		If no, reschedule visit. If yes, state if subject does not use products routinely or if stopped 48 hours prior to visit
Has Medical history been checked? complete medical history form	☐	☐	Dentist to initial	
Has Inclusion and Exclusion criteria been checked? **complete inclusion/exclusion sheet**	☐	☐		If not suitable, specify reason
Has Concomitant medications been checked? Record any conmeds taken within 30 days of the screening visit**.**	☐	☐		If none, state none
Has written Informed Consent been given by subject?	☐	☐		Record time consent taken here
Allocate a subject ID number After subject has given informed consent	☐	☐		State ID number here
<u>Unstimulated salivary collection</u> Collection over <u>5 mins</u>:	☐	☐		Seat subjects in a quiet, comfortable position, with head tilted forward (saliva collects to the front of the mouth). Ask subject to swallow to clear their mouth of any residual saliva at the start. Ask

Weight of empty bottle(g):_____ Weight of bottle+saliva (g):_____ Volume collected (g): _____ Assume that 1g = 1ml Flow rate (ml/5ml) = _____				subject to spit or dribble all excess saliva into a collection bottle over 5 mins. Ask subject not to drink, chew or speak until audible alarm sounds. Ask subject to spit all remaining saliva collection into the saliva collection bottle at the end of the 5 mins collection period. **UNSTIMULATED Salivary** **flow rate (ml/min)**_____ **NB - Unstimulated Salivary flow rate ≥ 0.25 ml/min to be suitable for study**

	YES	NO	STAFF INITIALS	COMMENTS (eg if not done)
<u>Stimulated salivary collection</u> Collection over 5 mins: Gum base chewing (1 min): Saliva collection (5 mins): Record time started: - _____ Weight of empty bottle(g):_____ Weight of bottle+saliva (g):_____ Volume collected (g): _____ Assume that 1g = 1ml Flow rate (ml/5ml) = _____	☐ ☐	☐ ☐		Ask subject to chew on gum base for one minute. After one minute, ask subject to swallow any pooled saliva. Ask subject to continue chewing the gum base for a further 5 minutes during which they will spit or dribble all excess saliva into a collection bottle. Ask subject not to drink, chew or speak until audible alarm sounds. Ask subject to spit all remaining saliva collection into the saliva collection bottle at the end of the 5 mins collection period. **STIMULATED Salivary** **flow rate (ml/min)**_____ **NB - Stimulated Salivary flow rate ≥ 0.8 ml/min to be suitable for study**

Is subject eligible to participate in the next part of the study?	☐	☐	Dentist initial	**Only if salivary flow rate is according to inclusion/exclusion criteria. If salivary flow is not, do not enter subject in study – <u>complete withdrawal form</u>**
DMFT / DMFS measurement (**complete using BASCoD criteria**) and Oral soft and hard tissue examination	☐	☐		**complete dental examination form**
Have impressions been taken of upper arch?	☐	☐		Tick “No” if subject already has an appliance from a previous study (TBC)
Does subject already have an appliance from a previous study that is suitable for use in this study? **TBC**	☐	☐	N/A ☐	
Does appliance need adjusting? **TBC**	☐	☐	N/A ☐	
Demographics (DOB, gender, race) and other personnel information recorded **– complete personnel information sheet**	☐	☐		
Has subject been given updated appointment card with appointment of next visit?	☐	☐		**Record date of next appointment:** _____

8.18 Appendix 18 Calculation of the amount of each therapeutic product during the experimental phases (table 8.20)

Table 8.20 The calculation of the amount of the therapeutic products (g) used at the end of each the randomised treatment phases

Amount of the therapeutic products (g) used during the randomised treatment phases												
		Treatment (A)		Treatment (AE)			Treatment (B)		Treatment (C)		Treatment (CD)	
Person code	Phase number	Elmex toothpaste	Phase number	A Elmex TP	E Elmex MR	Phase number	Non-fluoride toothpaste	Phase number	Pronamel toothpaste	Phase number	C Pronamel toothpaste	D Pronamel MR
001	5	36.72	1	32.5	284.6	4	36.2	2	54.2	3	59.8	297.2
002	2	9	3	4.48	239.1	1	12.31	4	16.5	5	27.2	338.9
003	3	20.9	2	27.18	213.5	5	19.2	4	27.3	1	38.2	246.6
004	5	28.1	3	30.9	213.8	2	35	4	45.5	1	31.1	246.4
005	2	28.17	3	34.08	257.5	4	38.9	1	48.6	5	40.8	243
006	4	28.41	5	28.0	284.8	2	41.92	3	52	1	68.4	205
008	2	23.7	1	21.6	372.4	5	20.7	4	19.3	3	17.2	318.6
009	4	20.3	2	21.7	318.3	3	14	5	43.5	1	16.2	270.3
010	3	34.2	5	24.2	258.3	1	29.3	2	50.6	4	29.1	203.1
011	4	20.4	3	16.3	257.6	5	25.1	2	30	1	24.2	243
012	4	28.68	3	18.2	314.6	1	6.72	2		5	36.7	206.6
013	1	16.88	3	20.7	249.7	2	18.82	2	21.7	5	25.2	238.4
014	1	53.98	5	54.4	221.8	2	47.32	4	66.1	3	60.6	212
016	4	26.38	2	38.8	301.2	1	39,82	5	39,9	3	35.0	264.5
018	5	19.8	3	22.3	144.7	4	10.7	1	41.2	2	30.9	175
019	3	21.88	4	24.1	134.3	2	21.6	1	18.1	5	46.8	220.7
020	3	35.88	2	47	242.9	4	29.58	5	42.8	1	57.12	355.6
Average usage		**27 g**		**27 g**	**253ml**		**25 g**		**38 g**		**38 g**	**252ml**

The table 8.18 illustrates the amount of each randomised therapeutic product used by the participant during the experimental phase which was calculated by the following equation:

The amount of the used product by the participant during the phase= the weight of the product given at the beginning of the phase – the weight of the product returned. Each therapeutic product was measured by balance measuring machine in the Dental Clinical Research Unit at the beginning and at the end of each of experiment phase

8.19 Appendix 19 Case Processing Summary related to the *in situ* study

Table 8.21 Summary of the total number of valid slabs among treatment groups after the experimental phases during *in situ* study

Case Processing Summary							
Treatment		Cases					
		Valid		Missing		Total	
		N	Percent	N	Percent	N	Percent
Erosion Plus abrasion	Group A	34	100.0%	0	0.0%	34	100.0%
	Group B	34	100.0%	0	0.0%	34	100.0%
	Group C	34	100.0%	0	0.0%	34	100.0%
	Group D	34	100.0%	0	0.0%	34	100.0%
	Group E	34	100.0%	0	0.0%	34	100.0%

8.20 Appendix 20 pH values of the therapeutic product after mixing with day artificial saliva to form a slurry formulation

Table 8.22 Therapeutic products and their pH values after mixing with day artificial saliva in study 2

Treatment	pH of the slurry
Elmex® toothpaste/ artificial day saliva slurries	5.7
Elmex® mouthrinse	4.1
Elmex® combination	6.7
Pronamel® toothpaste slurries	6.9
Pronamel® mouthrinse alone	6.3
Pronamel combination	6.4
GC tooth mousse	6.0
Non-fluoride® toothpaste slurries	6.9

8.21 Appendix 21 Readings of enamel slabs

Readings of the bovine enamel slabs to check the validity

	E3 after 7 days	E3 after 14 days	E3 after 21 days	E3 after 28 days
slab1	1.3	1.1	2.53	3.78
slab2	2.6	1.24	3.28	4.59
	E3 after 7 days	E3 after 14 days	E3 after 21 days	E3 after 28 days
slab1	1.24	2.41	3.18	4.49
slab2	2.1	2.08	2.97	4.15
	E4 after 7 days	E4 after 14 days	E4 after 21 days	E4 after 28 days
slab1	2.67	2.41	2.73	3.71
slab2	1.89	2.28	3.11	3.34
	E12 after 7 days	E2 after 14 days	E2 after 21 days	E2 after 28 days
slab1	1.09	3	3.56	5.65
slab2	1.22	3.76	3.79	5.03

Readings of the human enamel slabs to check the validity

	E1 after 7 days	E1 after 14 days	E1 after 21 days	E1 after 28 days
slab1	3.28	4.02	5.45	6.16
slab2	2.98	4.52	5.23	6.9
	E2 after 7 days	E2 after 14 days	E2 after 21 days	E2 after 28 days
slab1	1.22	2.55	3.34	4.02
slab2	1.3	2.71	3.01	3.05
	E3 after 7 days	E3 after 14 days	E3 after 21 days	E3 after 28 days
slab1	1.17	3.58	5.91	6.89
slab2	1.02	4.23	6.01	7.31
	E4 after 7 days	E4 after 14 days	E4 after 21 days	E4 after 28 days
slab1	1.38	4.44	3.55	4.98
slab2	2.23	3.89	3.2	4.09
	E12 after 7 days	E2 after 14 days	E2 after 21 days	E2 after 28 days
slab1	3.56	4.57	6.4	6.61
slab2	3.77	3.87	7.34	7.87

Appendix 21 ORCA abstract (F ALI) 097 (Conference poster)

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The Effect of Therapeutic Products in Combination on Prevention of ooth Surface Loss

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Aim: To study the effect of specialised fluoride toothpastes, mouthrinses and other remineralising agents specifically marketed for protection against sensitivity and/or erosion in combination on the surface loss of bovine enamel in vitro.

Methods: 60 enamel specimens, standardised for hardness, were mounted in acrylic blocks. They were divided into four experimental groups of 15 samples each:

1,400 ppm F as AmF followed by a F and stannous chloride containing rinse 2 times/day (Elmex TP plus MR); 1,450 ppm F as NaF and NaF 450 ppm F mouthrinse 2 times/day (PronamelTP plus MR); 1,450 ppm F as NaF + GC tooth mousse once per day (Pronamel TP plus TM) and 0 ppm F control. pH cycling was achieved using citric acid (0.3%, pH 3.6) for 2 min 5 times/day followed by 1 h in artificial saliva (pH 6.8). Slabs were subjected to 2 min brushing abrasion twice per day with 1: 3 slurry

of toothpaste/saliva at 300 g load. At all other times the samples were incubated in artificial saliva. Enamel loss was assessed by profilometry after 7, 14, 21 and 28 days. **Results:** Normality was checked and data analysed by One-way ANOVA and Bonferroni test. Significantly less surface loss (µm) was observed ($p \leq 0.005$)

with Elmex TP plus MR (0.40 ± 0.23) and Pronamel TP plus MR (0.60± 8 0.28) compared with Pronamel TP plus TM (2.56 ± 1.61) and 0 ppm F control (1.87± 8 1.84). **Conclusions:** In this model a combination of specialised toothpastes formulated to protect against erosion in combination with mouthrinses significantly decreased the effect of erosion and tooth brushing abrasion. We feel that a regimen comprising a combination of anti-erosion products might be

8.22 Appendix 22 EAPD abstract 2010, Harrogate, Leeds, UK.

An assessment of the protective effect of fluoridated toothpastes on enamel surface loss in vitro

Aim: The aim of this in vitro study was to assess anti-erosive potential of toothpastes containing different fluoride delivery systems on both bovine and human dental enamel subjected to both acidic erosion and tooth brushing abrasion.

Methods: Enamel specimens were mounted in acrylic blocks and flattened, tested for flatness by scanning profilometry (Scantron) and standardised for hardness using Knoop hardness. Slabs were then divided into five experimental groups: Meridol (amine+stannous fluoride, 1400ppm), Elmex *anti-caries* (amine fluoride 1400ppm), Pronamel (sodium fluoride, 1450 ppm), Elmex Sensitive plus, (amine fluoride 1400ppmF) and Aronal (fluoride-free). De/remineralisation cycling procedures were achieved by citric acid (0.3%, pH 3.6) for 2 mins followed by 1hr in artificial saliva (pH 6.8) 5 times daily for 28 days. All groups were subjected twice a day for 2 min brushing abrasion during application of slurry of toothpaste/saliva (1:3) with 15 strokes at 300g load. At all other times the samples were incubated in artificial saliva (pH 6.8). Mineral loss (µm) was assessed by profilometry scanning at 7, 14, 21 and 28 days.

Results: After 28 days of cycling, all groups showed enamel surface loss (µm). One-way ANOVA, demonstrated that Elmex sensitive plus (1.95±1.01µm), Elmex *anti-caries* (2.09±1.12µm), Pronamel (2.96±1.72µm) showed significantly lesser surface loss ($P<0.05$) than the control (6.13±3.03µm). Meridol (8.90±2.94µm) showed significantly more loss than all other groups.

Conclusions: In this model the effect of erosion and tooth brushing abrasion was decreased by toothpastes containing amine fluoride and sodium fluoride, whereas no protection was observed with the toothpaste containing amine plus stannous fluoride.

Key words: dental erosion, tooth brushing abrasion, fluoridated toothpastes.