IN VITRO AND IN SITU STUDIES TO INVESTIGATE THE EROSION OF HUMAN DENTAL TISSUES

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(MY WIFE, MY SON, AND MY PARENTS)

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ABSTRACT

Dental erosion is defined as irreversible loss of dental hard tissue by a chemical process that does not involve bacteria (Pindborg, 1970). The methods to study erosion and tooth surface loss are still not well developed. Also, the effect of various preventive agents is controversial.

Part-1: We wished to develop a standard methodology to create erosive lesions and to investigate them *in vitro*. Four methods (Knoop microhardness, surface profilometry, scanning electronmicroscopy, and confocal laser microscopy) were initially tested for their sensitivities to evaluate erosive lesions using a cycling regime of seven days. The enamel and dentine slabs were immersed under static conditions for 2 minutes, five times daily in fresh 200 ml aliquots 0.3% citric acid (pH 3.6). The slabs were incubated overnight and between erosive challenges in artificial saliva at 37°C. At the end of the cycling period, the slabs were analysed with the scanning profilometer (Scantron Proscan 2000) to measure the amount of surface loss. All of the methods showed an acceptable sensitivity, but surface profilometry was chosen as the preferred method mainly as it was reproducible and a user-friendly methodology.

Three different erosive products were investigated: orange juice, Sprite, and citric acid solution using a similar cycling regime and citric acid was then chosen to create erosive lesions for the remainder of this project.

The same cycling regime was repeated using three toothpastes containing different fluoride concentrations (0 ppm F, 1100 ppm F, 1385 ppm F). Each group was dipped with one of the toothpastes twice daily morning and evening, for two minutes each time. Fluoride toothpaste showed superiority over placebo for both enamel and dentine.

Part-2: In this part we concentrated on developing our *in vitro* cycling regime for the longitudinal study of erosion as well as investigating the effect of preventative agents on the reduction of tooth surface loss. A 21 day pH cycling regime was considered acceptable.

In a study which compared different concentrations of fluoride toothpaste (0 ppm, 250 ppm, 500 ppm, 1150 ppm, 1450 ppm) it was observed that the amount of tooth surface loss decreased with the increase of fluoride ion concentration in toothpastes. This was

statistically significant ($p \leq 0.05$) when placebo was compared to high fluoride concentration toothpastes (1100 ppm and 1450 ppm) for enamel, and significant between all fluoride toothpastes versus placebo for dentine.

The role of Relative Dentine Abrasivity index (RDA) was studied using our cycling regime using three different RDA toothpastes (40, 140, 160). There were no differences between toothpastes and the role of RDA remained unclear particularly when dentine specimens were used.

A comparison of the effect of toothpastes with different fluoride concentrations on tooth surface loss of human deciduous and permanent enamel *in vitro* showed a significantly lower surface loss when fluoride toothpastes were used for both deciduous and permanent enamel. In addition, deciduous enamel showed more surface loss than permanent enamel but this was not significant in all groups.

Part-3: This was a prospective randomised, double-blind, controlled in situ study. The effect of fluoride in toothpaste on tooth surface loss was investigated. Forty subjects participated and were fitted with an intra-oral removable palatal appliance containing two enamel and two dentine slabs. A pH cycling regime of 21 days was used which was similar to our developed *in vitro* model. Subjects used one of the two study toothpastes, 0 ppm or 1450 ppm F, in sequence. Subjects brushed their own teeth only for one minute twice daily (am/pm) followed immediately by swishing for a further minute with the resultant toothpaste slurry with the appliance intra-orally. Results (mean±SD) showed less than half as much erosive wear occurred for both enamel and dentine when a currently marketed toothpaste was used compared to the placebo (Enamel: 11.45±14.93 μm vs. 25.29±24.71 μm; and Dentine: 16.01±18.31 μm vs. 33.81±29.20 μm respectively). This difference was statistically significant ($p \leq 0.05$) using ANOVA test. In this longitudinal *in situ* model surface loss of both enamel and dentine was significantly reduced with use of a fluoridated dentifrice.

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3D	Three Dimensional
%	Percentage
≥	Greater than or equal to
µg	Microgram
µm	Micrometer
AE	Adverse Event
am	Morning
BDM	Biostatistics & Data Management
°C	Degrees Celsius
Ca:P	Calcium to Phosphate Ratios
CLSM	Confocal Laser Microscopy
cm	Centimeter
cm^2	Square Centimeter
CRF	Case Report Form
CSMH	Cross Sectional Microhardness
d	Day
DMFS	Decayed, Missing and Filled Surfaces
DMFT	Decayed, Missing and Filled Teeth
e.g.	For example
EDX	Energy dispersive X-ray spectroscopy
ESEM	Environmental Scanning Electron Microscope
Etc.	etcetera
F	Fluoride
g	Gram(s)
GCP	Good Clinical Practice
GSK	GlaxoSmithKline
GSKCH	GlaxoSmithKline Consumer Healthcare
h	Hour(s)
ICH	International Conference on Harmonisation
i.e.	That is
KMH	Knoop Microhardness
L	Liter
min	Minute(s)
ml or mL	Milliliter
mm	Millimeter
mM	Millimole
Mol	Mole
pH	Acidity

pm	Evening
ppm	Part per million
rpm	Rotate per minute
SAE	Serious Adverse Event
SAP	Statistical Analysis Program
SD	Standard Deviation
SE	Standard Error
sec	Second(s)
SEM	Scanning Electron Microscope
SKMH	Surface Knoop Microhardness
SL	Surface Loss
SP	Surface Profilometery
SUSAR	Suspected Unexpected Serious Adverse Event
TSL	Tooth Surface Loss
Tx	Treatment
UK	United Kingdom
V	Volt
Vol	Volume
VS	Versus
Wt	Weight

1 INTRODUCTION

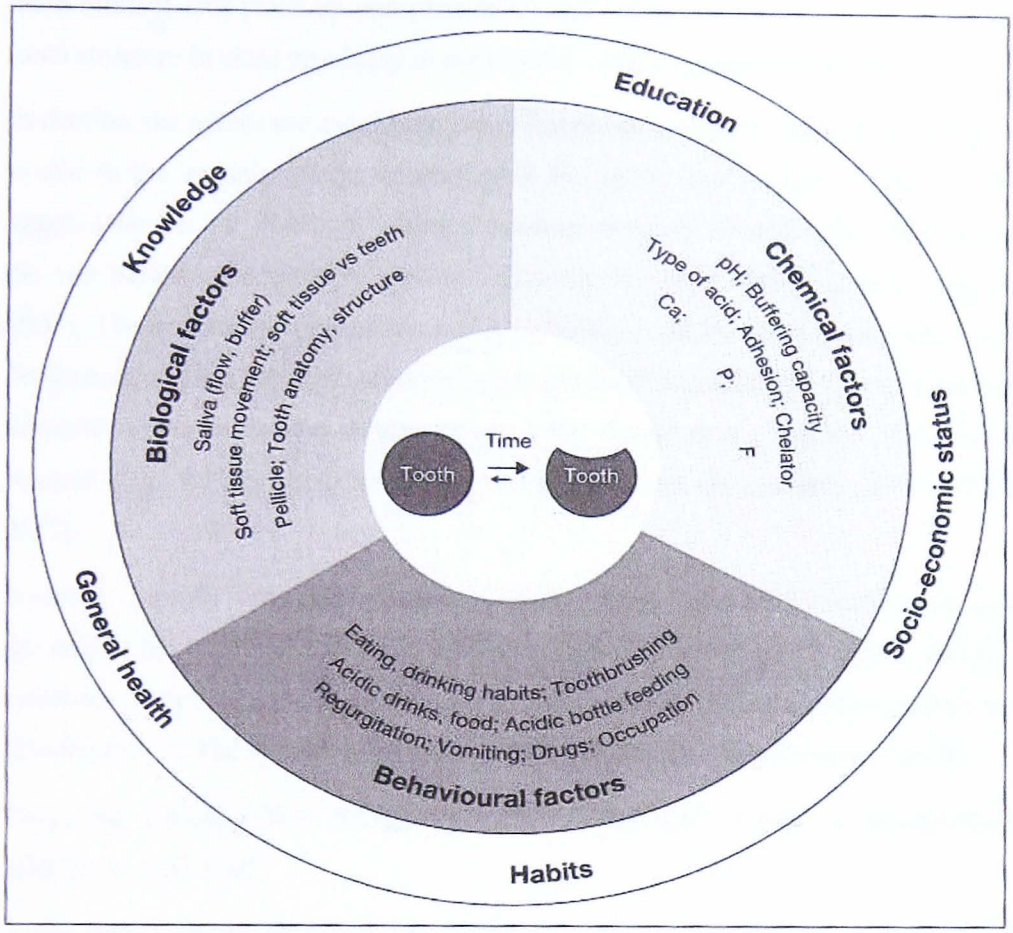
The main body of the literature review will cover the definition, chemistry, mechanisms, aetiology, prevalence, presentation and diagnosis of dental tooth surface loss.

1.1 Definition of Tooth Surface Loss

The cause of irreversible loss of tooth tissue includes abrasion (loss by wear of dental tissue caused by friction by foreign substances i.e. toothbrush, dentifrice), abfraction (loss of tooth surface at the cervical areas of teeth caused by tensile and compressive forces during tooth flexure), attrition (loss by wear of surface of tooth or restoration caused by tooth to tooth contact during mastication or parafunction), and erosion (progressive loss of hard dental tissues by chemical processes not involving bacterial action) (Imfeld, 1996; Bartlett and Shah, 2006) . The use of the term "tooth surface loss" to describe all these was proposed (Eccles, 1982) as it was difficult to determine the causal agents and there was often more than one process at work. It does not involve bacteria and is not directly associated with mechanical or traumatic factors or with dental caries (Lussi, 2006).

The most important issue for clinicians is the early recognition of dental erosion and identifying risk factors. Lack of awareness of the multifactorial nature of tooth wear (Figure 1.1) may lead to only partial treatment of the problem (e.g., an occlusal splint). Therefore, early recognition and initiation of preventive measures can prevent significant damage to the dentition.

Figure 1.1: Interaction of different factors for the development of erosive tooth wear (Courtesy of Lussi, 2006)



1.2 Mechanism of Dental Erosion (Chemistry of Dental Erosion)

Erosion affects the outer surface of enamel or dentine. Dentine is affected by erosion either after loss of enamel or as a process of gingival recession. This process is a complex process. The enamel surface in the oral environment is covered by a pellicle. This pellicle is an organic film free of bacteria derived mainly from salivary proteins and glycoproteins which cover the surface of teeth (Hannig *et al.,* 2005). Erosive solutions diffuse through this pellicle layer first and then interact with the mineral phase of the tooth, which is a carbonated and calcium deficient hydroxyapatite. Thereafter, acid with its hydrogen ion (or with its chelating capacity) will start to dissolve the crystal. First the prism sheath area and then the prism core are dissolved and create the honeycomb appearance (Meurman and Frank, 1991a). The un-ionised form of the acid will then diffuse into the inter-prismatic areas of enamel and dissolve mineral in the

subsurface region (Featherstone and Rodgers, 1981). 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 (Lussi and Hellwig, 2001).

In dentine, the events are even more complicated but remain the same in principle. This is due to the organic matrix which stalled the influx and outflow of demineralising agents (Hara *et al.*, 2005). In addition because of the physico-chemical differences of the two hard tissues, dentine is more vulnerable to erosion than enamel (Lussi *et al.*, 2006). The organic dentine matrix has a buffering capacity sufficient to retard further demineralisation. Therefore, chemical or mechanical degradation of the dentine matrix promotes demineralisation (Kleter *et al.*, 1994; Ganss *et al.*, 2004a). This process is stopped when no new acids and/or chelating substances are provided (Zero and Lussi, 2005).

Hydrogen ions (H^+) in acids or anions (chelating agents) that bind to calcium in enamel are behind the chemistry of dental erosion (Featherstone and Lussi, 2006). The H^+ ion combines with either the carbonate ion or the phosphate ion of enamel crystals which dissolves them. The following equation shows this process (Featherstone, 2000):

$$Ca_{10-x}\ Na_x\ (PO_4)_{6-y}\ (C0_3)z\ (OH)_{2-u}\ F_u + 3H^+ \rightarrow (10-x)Ca^{2+} + xNa^+ + (6-y)(HPO_4^{2-}) + z(HCO_3^-) + H_2O + uF^-$$

Acids such as citric acid have two different chemical mechanisms in dental erosion. When they are prepared in water they produce hydrogen ions, acid anions (e.g. citrate) and undissociated acid molecules. The amount of each of these components is determined by the acid dissociation constant and the pH of the solution. The hydrogen ion behaves as described above, the first chemical mechanism. The second chemical mechanism happens through the citrate anion which binds to calcium and removes it from the crystal surface (Featherstone, 2000).

1.3 Aetiology

Erosion may be either extrinsic or intrinsic in origin (Milosevic, 1998) or by a combination of extrinsic and intrinsic acids (Zero, 1996; Lussi *et al.*, 2006). It is essential that the aetiology of erosion is identified as the clinical management of the patient is based on management of the aetiological factors before definitive restorative care is undertaken.

Intrinsic erosion is caused by gastric acids. The main intrinsic acidic sources are:

1. Gastro oesophageal reflux.
2. Vomiting: spontaneous or self-induced and may be associated with a variety of medical conditions.
3. Rumination.

Extrinsic erosion is caused by extrinsic acids sources which may be:

1. Environmental.
2. Dietary.
3. Medication and oral hygiene products.
4. Lifestyle.

In addition, there are many predisposing factors which interact with erosive tooth wear (Lussi *et al.*, 2004a):

1. Chemical factors:
   - pH and buffering capacity of the product.
   - Type of acid (pKa values).
   - Adhesion of the product to the dental surface.
   - Chelating properties of the product.
   - Calcium concentration.
   - Phosphate concentration.
   - Fluoride concentration.
2. Behavioural factors:
   - Eating and drinking habits.
   - Healthier lifestyle: diets high in acidic fruits and vegetables.
   - Excessive consumption of acidic foods and drinks.
   - Night-time baby bottle feeding with acidic beverages.
   - Oral hygiene practices.
3. Biological factors (Mandel 1987):
   - Saliva: flow rate, composition, buffering capacity, stimulation capacity.
   - Acquired pellicle: diffusion-limiting properties and thickness.
   - Tooth composition and structure (e.g. fluoride content as FHAP or CaF_2-like particles).
   - Dental anatomy and occlusion.
   - Anatomy of oral soft tissues in relationship to the teeth.

- Physiological soft tissue movements.
- Polishing.

1.3.1 Intrinsic Acidic Sources

Gastric acid enters the mouth secondary to gastro-oesophageal reflux, vomiting or rumination.

1.3.1.1 Gastro-oesophageal reflux disease (GORD)

This is a common factor associated with dental erosion and may affect 7% of the adult population daily (Colin-Jones, 1996). The most common symptom of GORD is "heartburn". GORD is known to cause erosion in susceptible patients and should always be considered a possible cause of dental erosion. GORD is less of a problem in children.

1.3.1.2 Vomiting

Vomiting is either spontaneous or self-induced. It is often associated with an underlying medical condition. The other form of vomiting is self-induced vomiting. It causes purging and weight loss in the eating disorders of anorexia and bulimia nervosa.

1.3.1.3 Rumination (Voluntary Regurgitation)

Childhood neglect, abuse and other psychosocial stressors can precipitate rumination in children (Milosevic, 1998):

"The gastric refluxate is often held in the lower buccal pouch, either uni- or bilaterally, before being swallowed again, causing erosion of the adjacent buccal sites of canines and premolars as well as the typical palatal distribution".

1.3.1.4 Pregnancy Morning Sickness

Nausea and vomiting are common in the early stages of pregnancy (Milosevic, 1998).

1.3.2 Extrinsic Acidic Sources

1.3.2.1 Drinks

Dental erosion associated with the consumption of soft drinks, particularly in young age groups, is common (Dugmore and Rock, 2004; Lussi *et al.*, 2004a).

Carbonated beverages, fruit juices including so-called smoothies and fruit-flavoured mineral waters are tangy or refreshing because of their acidity and that makes them potentially erosive.

1.3.2.2 Foods

Fresh fruit, particularly citrus fruits, have erosive potential as do foods containing vinegar (as in crisps, salad dressings and pickles). Less well known is the influence of covert acids in food stuffs that have been associated with erosion in teenagers e.g. brown sauce, crisps, and ketchup (Milosevic *et al.*, 2004). Whether the erosive potential from these food sources is significant probably depends on individual eating habits (Milosevic, 1998).

1.3.2.3 Medication

Many medications induce a dry mouth and some induce nausea and vomiting. In addition some medications are acidic. This potential co-morbidity has not been investigated widely.

1.3.2.4 Other Extrinsic Sources

Industrial electrolytic processes, such as galvanizing, metal plating and battery manufacture can release acid vapour into the work environment. This factor has been minimised by introducing industrial safety regulations (Gandara and Truelove, 1999).

1.4 Prevalence

Dental erosion were reported very early in the late 1800's and early 1900's and related consumption of acidic drinks to dental erosion and tooth wear (Al-Dlaigan *et al.*, 2001). However until the early 1990's, epidemiological investigations were undertaken to show the prevalence of dental erosion in the general population (Al-Dlaigan *et al.*,

2001). Prevalence data from cross-sectional UK studies indicated that dental erosion increased between different age cohorts of young people over time (Table 1.1 and Table 1.2).This increase included the amount of tissue loss of each surface, the surfaces involved of each tooth, and number of teeth involved. Dental erosion was associated significantly with increased soft drink consumption (Millward *et al.,* 1994; Milosevic *et al.,* 1997). Smith & Robb (1996) used a Tooth Wear Index (TWI) to record dental erosion in 1007 patients in England. This index records tooth wear by number and degree of involved tooth surfaces. It uses the degree of wear of dental tissues in certain age groups (adolescent through elderly). O'Sullivan (2000) developed TWI to be used for children in the adolescent group. In North America, the role of erosion in tooth wear is not as well understood or appreciated as it is in Europe (Bartlett *et al.,* 1999). Even within Europe the interpretation of erosive tooth wear differs. Some countries will include the cervical wear lesions as fundamentally erosive whereas others might consider abrasion more important. This in itself is not a problem provided that any measure used to evaluate tooth wear does not discriminate between the aetiology. However, there are a number of indices measuring specific tooth sites or surfaces, for example palatal surfaces of upper incisors and occlusal surfaces of lower molars, and using them to give data on prevalence.

1.4.1 Prevalence of tooth wear and erosion is the deciduous dentition

Most of the studies on tooth wear in children have been reported from Europe (Wiegand *et al.,* 2006). Millward *et al.,* (1994) investigated 178, 4-year old children from Birmingham, UK and reported that as high as 17% showed involvement of dentine exposure. The authors reported that almost half the subjects showed some sign of tooth wear and the most commonly affected tooth surface was the palatal/lingual of the maxillary incisors. Another study (Al-Malik *et al.,* 2002) on 987 pre-school children conducted in Saudi Arabia reported 31% showed some evidence of tooth wear with 13% having dentine exposure. However, in this study the measurements were restricted to the primary maxillary incisors. A larger study in China on 1,949 children aged 3-5 years old reported that only 5.7% showed signs of wear (Luo *et al.,* 2005). It is difficult to understand why the geographical areas showed such a difference but it may reflect the socio-economic status of the nations.

Table 1.1: Prevalence studies on dental erosion in the United Kingdom since 1994 (Courtesy Guidelines of dental erosions management, Leeds Dental Institute) (1" primary teeth. 2" secondary/permanent teeth).

AUTHOR	YEAR OF PUBLICATION	AGE	SAMPLE SIZE	Erosion Prevalence (%)	% WITH PALATAL/ OCCLUSAL/ LABIAL DENTINE EXPOSED	TEETH
Millward *et al.*,	1994	4-5	178	50%		All 1" teeth
Milosevic *et al.*,	1994	14	1,035	30%	8	All 2" teeth
Nunn *et al.*,	1995	12-13.5	135	68%-95%		U1" Incisors
Smith & Robb	1996	<26->65	1007	26% with extensive TW		All 2" teeth
Al-Dlaigan *et al.*,	2001	14	418	100%		All 2" teeth
Dugmore & Rock	2004	12	1,753	56.3%-64.1%		Incisors & First Molars
Bardsley *et al.*,	2004	14	2,351	53%	10	All 12 anterior and occ of first molars

Table 1.2: Prevalence studies on dental erosion in Europe (Courtesy Lussi, 2006).

AUTHOR	YEAR OF PUBLICATION	AGE	SAMPLE SIZE	Erosion Prevalence (%)
Jaeggi and Lussi (Swiss)	2004	5-9	42	14.3%
Caglar *et al.*, (Netherlands)	2005	11	153	28%
Ganss *et al.*, (Germany)	2001	8-14	1000	11.8%
Truin *et al.*, (Netherlands)	2005	12	324	24%
Arnadottir *et al.*, (Iceland)	2003	15	278	21.6%
Van Rijkom (Netherlands)	2002	10-13 15-16	345 400	3% 30%
Larsen *et al.*, (Denmark)	2005	15-17	558	14%
Jaeggi *et al.*, (Switzerland)	1999	19-25	417	up to 82% in enamel
Schiffner	2002	35-44 65-74	655 1027	42.1% 46.3%

1.4.2 Prevalence of tooth wear and erosion in adolescents

There are studies undertaken in the mixed dentition of children at school. One group of researchers measured erosive wear on study models/casts of 1000, 11-year olds and reported up to 70% of tooth surfaces and 26.4% with advanced lesions of erosive wear (Ganss *et al.*, 2001). This finding was not consistent with the finding in a smaller sample of 210, 11-14 year olds and observed less destruction with less than 2% with dentine exposure (Bartlett *et al.*, 1998). Another difference between these two studies was that the most commonly worn surface in the study reported by Bartlett *et al.*, was the palatal surfaces whilst Ganss *et al.*, reported the occlusal and incisal surfaces. Truin *et al.*, (2005) reported the prevalence of erosion in a group of 12 year old children in the Netherlands. Their examination was limited to the palatal surfaces of the incisors and canines and the occlusal surfaces of first molars. Wear was observed in 59.7% of the subjects with 2.7% having dentinal involvement. Milosevic *et al.*, (1994) reported 30% dentinal exposure in 1035, 14-year olds in Liverpool, England. Their study included all tooth surfaces and the most commonly affected surfaces were the incisal edges of upper and lower incisors. Bardsley *et al.*, (2004) showed even a higher prevalence of dentine exposure approaching 50%. These results have been supported by other studies in England. Al-Dlaigan *et al.*, (2001) reported 51% of subjects with dentine exposure, however only 2% had severe levels. Dugmore and Rock (2003) reported lower levels of dentine involvement with only 2% from 1,753, 12-year olds. It is difficult to understand why such a wide variation in dentine exposure is observed in so many different studies. A recent review concluded there was an increasing trend towards increasing wear with age (Jaeggi and Lussi, 2006). In addition, dietary habits, presence of gastro-oesophageal reflux and socio-economic status all affected the prevalence of erosive tooth wear.

1.4.3 Prevalence of tooth wear and erosion in adults

Lussi *et al.*, (1991) reported around 10% of 391 subjects had exposed dentine. Xhonga and Valdmanis (1983) examined 527 subjects selected randomly and aged between 14 and 88 years. The authors suggested that the prevalence in the USA was around 25% but dentine involvement was comparatively rare at 4%. Xhonga and Valdmanis (1983) in another clinical study in general dental practice on 1007 adults aged 18-88 reported pathological levels of wear approaching 5%.

1.5 Presentation and Diagnosis

Few studies have investigated the site specificity of dental erosion but most reports indicate that the incisal, palatal and occlusal surfaces are commonly affected with buccal or labial surfaces also being involved (O'Sullivan and Milosevic, UK guidelines). The stages are:

1. Enamel becomes thinner creating chamfered ridges or ledges.
2. Cupped cusp tips and grooved incisal edges.
3. Dentine exposure which causes incisal chipping and teeth may appear darker.
4. Main complaints are aesthetics and sensitivity.

The most reliable technique to measure diseases of teeth in large populations is indices. Most indices use changes to the anatomical appearance of teeth to record the amount of wear. Some indices measure tooth wear on every surface of every tooth (Smith and Knight, 1984), some use selected sites (O'Brien 1994) and others use specific surfaces (Dahl *et al.*, 1989). Other studies have reported the prevalence of erosion rather than tooth wear (Johansson *et al.*, 1993). The challenge is to diagnose the aetiology from the appearance of a lesion without a comprehensive dietary and dental history (Kidd *et al.*, 1993; Bartlett *et al.*, 2000). In most cases changes in the anatomy of teeth from tooth wear is a combination of erosion, abrasion and attrition and it is difficult to assess which component is most important. The most cited examples of erosion indices developed during the last 20 years (Ganss & Lussi, 2006) are:

1. The Eccle's Index (1979)
2. The Smith and Knight Tooth Wear Index (TWI) (1984)
3. Modified scoring system of Linkosalo and Markkanen (1985)
4. Aine Index 1993
5. Erosion Index according to Lussi (1996)
6. UK National Survey of Children's Dental Health Index (1999/2003)
7. The Larsen and Westergaard Index (2000)
8. The O'Sullivan Index (2000)

Most indices use different clinical examination standards for measuring tooth erosion especially in pre-school and school children (Jaeggi & Lussi, 2006). Such examination standards could be:

1. Full mouth or partial recording
2. Examination of primary and/or permanent teeth

3. Examination of all surfaces or partial recording of surfaces

The validity and reliability of these indices are questionable and needs to be reviewed (Berg-Beckhoff *et al.*, 2008). These indices are not comparable and meta analysis is not possible because:

1. The criteria to record the grading for erosion differed.
2. Most of the indices used are based on the clinical severity of erosion, focusing on accessible teeth but not all teeth.
3. Some indices score tooth wear in general irrespective of its predominant aetiology.
4. Most of the indices do not have validation data.

1.6 Prevention

Acids can act on the tooth structure either exogenously, for example on consumption of acidic foods and drinks, or endogenously due to gastric acid. Depending upon the individual predisposition, acid-induced losses of tooth structure become clinically evident only on frequent chronic action over a longer period.

Primary prevention should consist generally of appropriate information about causes and avoidance of erosive tooth damage within the scope of the established prevention strategies and individual counselling. Further measures related to the population depend upon the prevalence of erosive losses of tooth structure and should therefore be discussed specifically for the country in question. Secondary prevention comprises above all the early and differential diagnostically correct detection of the early stages of erosions within the scope of screening examinations and individually coordinated causal measures.

Understanding the pathogenesis and ultrastructure of erosions allows us better understanding of prevention and therapy strategies. In erosive demineralisation there is centripetal loss of substance in the enamel, which is manifested as clinically visible surface defect in the case of continuous exposure to acids. A partially demineralised zone with reduced microhardness is found on the eroded enamel surface (Lussi *et al.*, 1995), which corresponds ultrastructurally more or less to a classical etching pattern (Meurman and Frank 1991b). Thus, the ultrastructure of enamel erosion differs fundamentally from initial enamel caries, in which the zone of the greatest demineralisation lies under a pseudo-intact surface layer (Thylstrup and Fejerskov

1994). In the dentine after short-time action of acids, there is firstly loss of mineral in the region of the peritubular dentine and with longer action time enlargement of the dentin tubules with demineralisation of the intertubular dentine (Noack 1989, Meurman *et al.*, 1991). In this case the organic matrix is exposed. Both in laboratory experiments *(in vitro)* and in samples which were worn in the mouth (*in situ*), this organic covering layer is displayed regularly but the role that it plays clinically is so far unclear. In contrast to caries, which as a rule always requires invasive therapy as from a certain stage, acid-induced tooth structure defects come to a standstill independently of their extent, if sufficient causal or symptomatic measures are adopted. As a rule no restorative treatment is then necessary, unless there are aesthetic or functional impairments.

The causal therapy of acid-induced tooth structure defects starts with the identification of the acid source. This includes an anamnestic consultation which includes questions regarding exogenous and endogenous acid exposure. In addition, an open nutrition protocol (Lussi 1996) can give further indications in many cases with regard to quantity, nature and frequency of acid action due to food. In the case of exogenous acid action, the causal therapy consists in changing the eating habits, which frequently does not necessarily require major changes in behaviour. For example, apart from reducing the frequency of consumption, less erosive drinks can be consumed. Both *in vitro* and *in situ* studies have shown that solely the addition of calcium can reduce considerably the erosive potential of drinks (Hughes *et al.*, 2000). Fruit can be consumed together with milk products. In the case of endogenous acid action, medical treatment can be indicated (e.g. in reflux diseases), but, frequently causal therapy is difficult. For example, eating disorders with chronic vomiting can exist for years despite therapeutic efforts. In these cases symptomatic measures are necessary just as in unclarified exposure to acids.

The purpose of symptomatic measures is to modify the tooth surface so that the erosive demineralisation and thus the loss of microhardness are reduced.

Substances that lead to acid resistant mineral precipitations in or on the tooth surface or that form permanent coatings are suitable for this. The application of dentine adhesives has been discussed as non-mineral coating (Azzopardi *et al.*, 2001; Sundaram *et al.*, 2007). This measure is suitable as an acute measure. Since it can be expected that these

coatings are abraded at least in the medium term, their protective effect may be limited in time.

Mineral precipitates can be expected generally from oversaturated calcium/phosphate solutions such as saliva. Therefore, it is frequently recommended not to clean the teeth directly after the action of acids but to wait for the "remineralisation" of the tooth surfaces. Such recommendations were derived from laboratory studies with saturated/oversaturated calcium/phosphate solutions. However, *in situ* experiments have been able to prove only slight effects of waiting times (Jaeggi and Lussi 1999; Ganss *et al.*, 2007a). There is a good explanation for these apparently contradictory findings. *In vitro* the precipitation of calcium and phosphate from saturated solutions onto etched enamel depends upon different factors (Amjad *et al.*, 1981), but crystal growth can be proven easily. However, intraorally the oversaturation of the saliva is maintained by proteins such as statherin, proline-rich proteins or histidine-rich polypeptides, so that normally no precipitation of calcium/phosphate salts takes place on clean tooth surfaces. Mineralisation processes can occur only if diffusion of these proteins is obstructed, as for example by the pseudo-intact surface layer of initial caries (remineralisation of the initial caries) or by plaque (formation of calculus). In the case of eroded enamel under oral conditions neither a relevant increase of the microhardness has been proven (Collys *et al.*, 1991; Collys *et al.*, 1993) nor could the precipitation of mineral be shown (Garberoglio and Cozzani 1979). These findings also correspond to the results of *in situ* studies that have shown only a slight effect of waiting times between erosive demineralisation and brushing (Jaeggi and Lussi 1999; Attin *et al.*, 2001a; Ganss *et al.*, 2007b). A change of the oral hygiene habits, apart from insufficient plaque control, is therefore expedient only in the case of traumatic oral hygiene techniques or extreme effects of acid.

However, mineral precipitates can be generated by the local application of fluorides. According to the form of administration, more or less pronounced covering similar to CaF_2 layers arise, which however are relatively easily soluble in acids. Contradictory estimates are published in the literature about the effectiveness of these measures (Wiegand and Attin, 2003). Therefore fluoriddidation recommendations that lead to the most pronounced possible precipitates are generally given. Precipitates similar to CaF_2 are thicker the more acidic and concentrated the fluoride products are, and the longer the action time is (Saxegaard and Rölla, 1988). Therefore acid preparations should be

used as frequently as possible as gels with high fluoride concentration and/or as mouth wash solutions in addition to a fluoride toothpaste (Ganss, 2005; Wiegand and Attin, 2003). In fact, intensive fluoriddidation can be very effective at least under *in situ* conditions even with longer and frequent effects of acid. Intensive fluoriddidation has also proven to be clearly more effective than waiting times for reducing abrasion by brushing (Ganss *et al.*, 2007b). However, such a therapeutic approach means that patients should fluoridate frequently and possibly with different forms of preparations such as mouth rinse and gel, which means a considerable organizational and financial effort. Such recommendations are therefore only limitedly suitable for longer therapeutic application and are not suitable for preventive measures.

So far the fluoride compounds of sodium fluoride, amine fluoride or sodium monofluorophosphate known from cariology and contained generally most frequently in oral hygiene products have been examined. However, more recent studies (Schlueter *et al.*, 2007; Ganss *et al.*, 2008) show that the effectiveness of fluorides in the context of erosions is determined essentially by the nature of the fluoride compound. The effectiveness of different fluoride compounds becomes especially clear if preparations of the same pH value and same concentration are compared with one another (Schlueter *et al.*, 2007; Ganss *et al.*, 2008). Thus it can be shown that erosive mineral losses can be prevented practically at least under mild conditions by stannous fluoride or amine fluoride/stannous fluoride solutions, whereas sodium fluoride or amine fluoride/sodium fluoride solutions appear to be significantly less effective. A new result so far is that even a stannous chloride solution without fluoride shows effectiveness that lies in the order of magnitude of a sodium fluoride solution. Electron optical examinations have shown that after application of solutions containing tin, apparently relatively acid-resistant precipitates are formed (Ganss *et al.*, 2008), whereby quite generally fluoride compounds with polyvalent metal ions come into view as potential erosion inhibitors. Titanium and, as already mentioned, tin must be emphasized specially in this connection (Ganss *et al.*, 2006; Hove *et al.*, 2007). Titanium fluoride has been examined so far in the form of experimental preparations. After treatment with titanium fluoride glaze-like deposits that are resistant to mechanical and chemical influences are formed and they can even withstand treatment with concentrated hydrochloric acid (BuyUkyilmaz *et al.*, 1997). The reaction mechanisms that lead to the formation of such deposits are not clarified, but reactions between the titanium ion and the organic constituents of the tooth structure or the oxygen available on the tooth surface as well as

the formation of stable titanium dioxide are discussed. So far the good effectiveness of titanium fluoride has been shown primarily for concentrated and very acid solutions that are not suitable for domestic use. However, more practicable forms of preparation appear to develop no better effect than, for example, solutions containing stannous fluoride. It can be stated in conclusion that in the symptomatic therapy with fluorides of erosions apparently the fluoride compound is significant.

1.7 Summary

The literature review in the prevention sections shows the effectiveness of fluoride application in preventing or reducing dental erosion. However, it is difficult sometimes to compare between these studies as they used different protocols and/or different erosive challenges. The aim of this research project was to develop a modified methodology that mimics oral environment to investigate preventative agents (i.e. toothpaste) and to use this methodology for further *in vitro* studies and eventually transfer this into clinical studies.

2 STUDIES TO DEVELOP METHODS TO INVESTIGATE DENTAL EROSION *IN VITRO*

2.1 Introduction

In this chapter, we studied different methods to investigate dental erosion, to develop erosive lesions or to investigate a modified erosive cycling technique *in vitro*.

2.1.1 Techniques for the production of dental eroded lesions *in vitro*

In vitro studies on dental erosion have tried to create erosive lesions on enamel and/or dentine using different methods. In most of these *in vitro* studies erosive lesions are created by simply immersing a tooth into the erosive challenge (i.e. citric acid) or soft drinks (i.e. orange juice) for a prolonged period of time. This might provide information on the erosive potential of these products; however it exaggerates the potential erosive effects due to the absence of factors present in the oral environment such as saliva remineralisation factor, salivary pellicle, and buffering capacity of saliva (Hunter *et al.*, 2000; Lussi *et al.*, 1995; Eisenburger and Addy, 2001). However, Amaechi *et al.* (1999a) used a modified technique to create dental eroded lesions. They immersed teeth continuously in stirred pure orange juice (20 mL/specimen) at regular intervals six times per day for 5 minutes on each occasion for a period of 24 days, giving 30 min daily exposure or a total of 12 hours of exposure to orange juice. The immersion was carried out at room temperature (approximately 20°C). In between exposures to orange juice and for the remaining 12 hours overnight, the teeth were either stored in artificial saliva (20 mL/specimen) or in de-ionised distilled water. These two groups were compared with prolonged exposure for 12 hours of a third group in pure orange juice. Mineral loss was measured using microradiography to compare the differences between groups. Mineral loss (Vol% mm) was significantly lower in those specimens cycled in orange juice and artificial saliva compared with those cycled in orange juice and de-ionized distilled water ($p \leq 0{\cdot}01$) and those from the single 12 hours immersion in orange juice ($p \leq 0{\cdot}01$). It was concluded that the modification technique for creation of dental eroded lesions using artificial saliva had reduced the potential erosive effect of orange juice. Our aim was to develop a methodology to study dental erosion *in vitro* in a situation close to the real life scenario. Therefore, a modification of the Amaechi *et al.* (1999a)

technique was employed in this elementary stage. This will be described in the methods section.

2.1.2 Dental erosion evaluation techniques

Many techniques have been used to investigate the loss of tooth substance during erosion (Barbour and Rees, 2004).

The study design (e.g. need for single measurement or for repeated measurements), the study model (e.g. use of natural teeth or intra-oral devices) and the method's specification (e.g. sensitivity, reproducibility) determined the study design selection. In addition, the available resources such as the available expertise, the availability of the equipment or the cost of purchase or construction, time restrictions and cost of measurements, play an important role in the selection of the appropriate evaluation technique (ten Bosch and Angmar-Månsson, 1991). Several quantitative techniques have been developed for this purpose (Barbour and Rees, 2004):

1. Surface hardness and nano-indentation techniques
2. Profilometry
3. Microradiography
4. Chemical analysis
5. Microscopy techniques (SEM, ESEM)
6. Confocal laser scanning microscopy (CLSM)
7. Atomic force microscopy (AFM)
8. Secondary ion mass spectroscopy (SIMS)
9. Quantitative light-induced fluorescence microradiography.
10. Micro CT (x-ray micro tomography).

2.1.2.1 Microhardnesss

Microhardness indentation measurements have been used to determine de- and remineralisation effects using the *in situ* model of Koulourides (1966). Microhardness testing measures the resistance of enamel surfaces to indenter penetration and is a function of the degree of porosity of the superficial enamel layer that indicates mineral loss or gain in subsurface lesions (Koulourides, 1971). In this method, a Knoop or Vickers diamond is positioned on the sample with a well-defined load for a given time, in order to create an indentation in the tooth surface. The indentation length is then

determined microscopically (in μm) (Angmar-Månsson and ten Bosch, 1991). The microhardness measurements are very sensitive to changes in mineral density (Featherstone and Zero, 1992), and can provide indirect evidence of mineral loss or gain.

2.1.2.1.1 Types of microhardness testing

There are two types of microhardness tests, surface microhardness and cross-sectional microhardness.

Surface microhardness (SMH): where a load with a diamond indenter is applied perpendicular to a polished tissue surface. SMH measurements, when used in the assessment of de/remineralisation, can only give qualitative information on mineral changes, and the samples must have flat surfaces (Arends and ten Bosch, 1992). Furthermore, phenomena such as lesion shape, mineral redistribution, and protein uptake *in situ*, may affect the indentation length values. A linear relationship between indentation length and lesion depth is valid only for a limited range of lesion depth values (Arends *et al.*, 1980; Zero *et al.*, 1990). This technique is a destructive and allows for a longitudinal study of the same specimen, however it cannot give details about the subsurface hardness changes or inform about any structural alterations to different sides of the lesion (Featherstone *et al.*, 1983).

Cross-sectional microhardness (CSMH) where the diamond indenter load is applied parallel to the tissue's anatomical surface (Arends *et al.*, 1980). CSMH experiments have the advantage that, indirectly, the mineral content can be determined quantitatively and the mineral loss and mineral gain values can be estimated. The mineral profile (volume percentage of mineral as a function of the distance from the outer surface) can also be obtained. A disadvantage is that the outermost 25 μm of a sample cannot be included in the measurement (Arends and ten Bosch, 1992).

Some researchers (Jaeggi and Lussi, 1999; Joiner *et al.*, 2004) tried to measure the amount of tooth surface loss caused by the erosive/abrasive challenge using microhardness. They measured the depth before and after abrasion. They were not able to measure the amount of surface loss because acids caused surface loss in the body of the indentation not only from its surroundings.

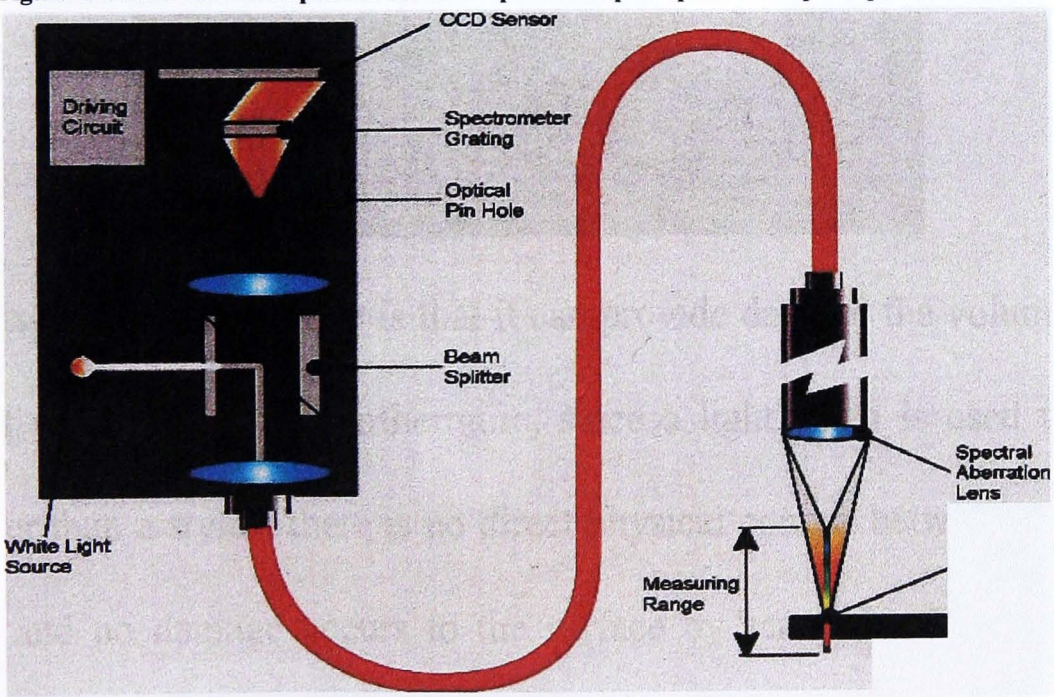
When using microhardness to evaluate the hardness of of dentine, a direct reading of the indent is a critical issue due to the elasticity of dentine. The length of indents changes as this phonemena (Hosoya, 2000).

The main advantages of microhardness are the low costs and the possibility of combining it with other methods.

2.1.2.2 Profilometry

Profilometry is a means of measuring surface loss of dental hard tissues. It uses a small metal stylus (20 mm it diameter) that scans across the enamel surface at a rate of around 10 mm/min for the acquisition, graphical presentation, evaluation and documentation of surface profiles. In this technique, the enamel surface is divided into two parts, an exposed and a covered part using nail varnish or tape. The sample surface is scanned before and after erosion, and the amount of material loss can be measured from the trace produced. Alternatively, a cast may be made of the eroded enamel surface and the profilometer used to measure the profile of the cast. More recently, non-contact profilometry has been used to assess tooth surface loss. In this technique, the traditional contact stylus is replaced with white light or a laser, and interferometry is used to build up a map of the surface (Figure 2.1).

Figure 2.1: Schematic description of the basic operational principles of the optical profilometer

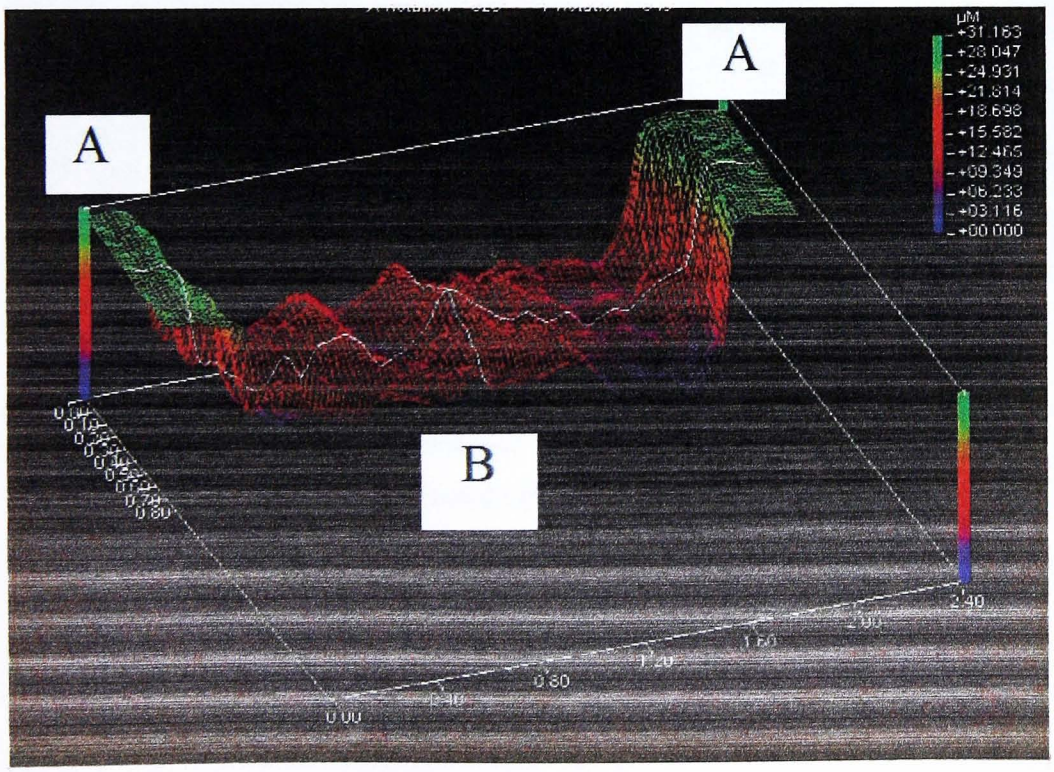


The optical profilometer transmits safe white light through a lens that has a spectral aberration built into it. This causes the white light to divide into the full spectral field, and each of the different colour frequencies are focused at a slightly different point through a defined measuring range. When an object is then placed within this range, only one particular colour frequency reflects back from the surface. This information is then passed back into a processor where a spectrometer analyses the signal and converts it into a measurement (Figure 2.2).

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. 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 is a quick and simple technique that can be used over a relatively large area of enamel. However, the enamel sample has to be ground flat prior to use. Furthermore, this technique is used for the more advanced stages of erosion than indentation techniques as it measures surface loss rather than surface softening

Figure 2.2: Erosive lesion on an enamel slab scanned by an optical profilometer. "A": are baswelines areas which are covered with nail varnish during experiment. "B": is the eroded area. Profilimetry measues the mean depth (vertical depth) of area "B" compared to area "A".

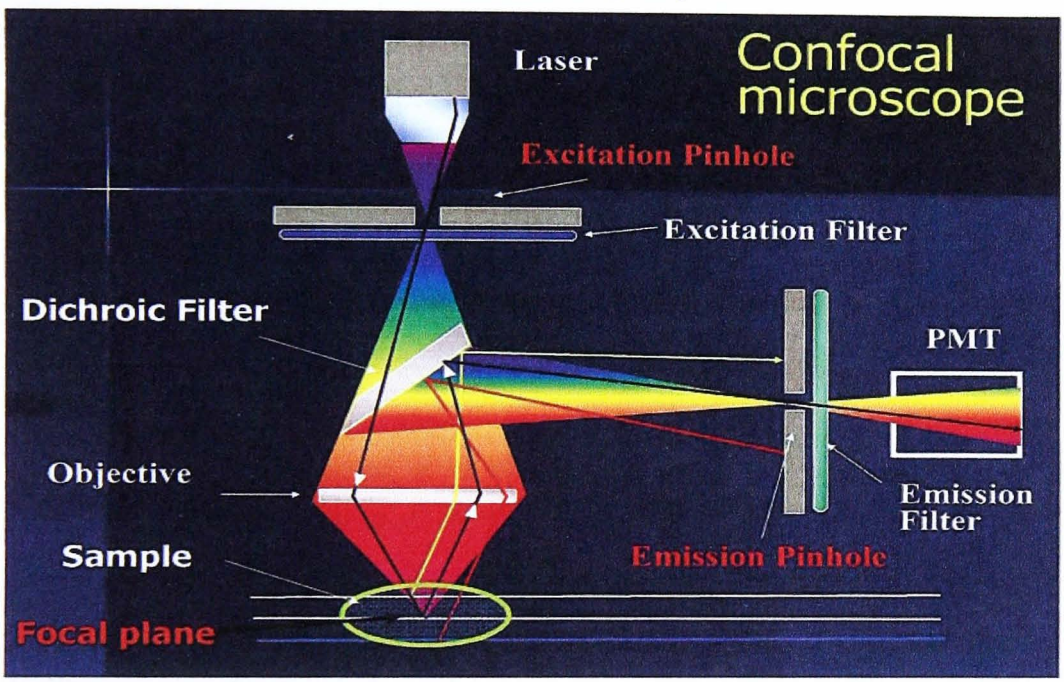


2.1.2.3 Confocal laser scanning microscopy

The first single-beam confocal laser scanning microscopes were developed in a number of laboratories and applied to biological and material specimens (Sheppard and Shotton, 1997).

Confocal microscopy derives use of an aperture in the conjugate focal plane of an objective lens in both the illumination and imaging pathways of a microscope. The areas surrounding the aperture reject stray light returning from areas which are not in the focal plane of the lens (Watson, 1997). Confocal laser scanning microscopy can be applied either by using reflection or fluorescence (Figure 2.3).

Figure 2.3: Schematic representation of the confocal principle



Excitatory laser light from the illuminating aperture is reflected by the dichroic mirror and is focused by the microscope objective lens to a diffraction limited spot at the focal plane within the 3D specimen. Reflected light (or fluorescence emission) is collected by the objective and passes through the dichroic mirror and the emission filter. Only those emissions from the in-focus areas are able to pass unimpeded through the confocal detector aperture to be detected by the photomultiplier. Light from regions below and above the focal plane have different primary image plane foci and are thus severely attenuated by the confocal aperture, contributing essentially nothing to the final image.

The theoretical resolution of a confocal system is primarily a function of the numerical aperture (NA, light gathering ability) of the optical system and the wavelength (λ) of the light. Reducing λ increases the resolution.

Confocal microscopy exhibits several advantages over conventional microscopy:

1. The out-of-focus blur is essentially absent from confocal images, giving the capability for direct non-invasive serial optical sectioning of intact and even living specimens. This leads to the possibility of generating three-dimensional (3D) images of thick transparent objects such as biological cells and tissues.

2. The contrast and sharpness of images are much more improved. Therefore the specimens should not be well-flattened or very thin as required for conventional microscopical techniques.

3. It has also got a small but significant improvement of the lateral resolution. It rejects light not only from the out-of-focus specimen planes but also light scattered from within the optical instrument itself, resulting in an increased contrast and signal-to-noise ratio of the final image.

4. Specimens do not require any special preparation and, therefore, are not subjected to distortions caused by dehydration needed for other procedures such as SEM (Fontana *et al.*, 1996).

5. The operator is not exposed to the risks associated with the usage of x -rays (in contrast with TMR) (Fontana *et al.*, 1996).

6. Confocal microscopy is also compatible with computer image storage techniques, allowing, for example, generation of high-resolution digitised data sets of the 3D distribution of labels within cells or tissue, or of the topography of a surface, suitable for subsequent image processing.

The applications of confocal microscopy in dental research have been gradually expanding in many areas, such as caries research (Fontana *et al.*, 1996); studies of toothbrush abrasion of enamel (Kodaka *et al.*, 1999); studies of bleaching effect of toothpastes (White *et al.*, 2003); investigations of soft and hard tissue responses to biomaterials and implants (Oakley and Brunnette, 1993); studies in dental materials (Watson, 1997).

2.2 Summary

From the preceding literature review, it is clear that erosion is a multi-factorial disease and simulating this *in vitro* is difficult. Therefore, it was felt timely to develop a cycling technique as close as possible to the in vivo situation for the creation of erosive lesions *in vitro*. In addition, by using the methodological techniques previously described to evaluate this cycling technique as well as to compare the sensitivity of those methods on measuring the changes caused by this cycling technique to help in the development of anti-erosion preventive agents containing fluoride.

The aim of this project was to standardise the methodology for the investigation of dental erosion of enamel and dentine and. In addition, to test this methodology in studying the role of fluoride incorporated into therapeutic agents (toothpaste) on tooth wear, in particular erosion.

2.3 Null Hypotheses

1. There are no differences in the reliability and user-friendly of the following methods in detecting changes on enamel hard tissues under citric acid erosive challenge: surface Knoop microhardness (SKMH), surface profilometry (SP), and confocal laser scanning microscopy (CLSM).
2. There is no difference between citric acid (0.3% pH 3.6), pure orange juice, or Sprite in creating erosive lesions.
3. There is no difference between three concentrations of fluoride toothpaste (0 ppm F, 1100 ppm F, 1385 ppm F) for the protection of dental hard tissues from erosive lesion creation.

2.4 Materials and Methods

This chapter describes the materials and methods used during the investigations. The investigations were divided into three studies for enamel and dentine:

Study-1: To check the reproducibility and the sensitivity of the four methods (Knoop microhardness "KMH", surface profilometer "SP", and confocal laser scanning microscopy "CLSM") selected for detecting the changes of eroded lesions created *in vitro* using 0.3% citric acid (pH 3.6).

Study-2: To compare three erosive products: 0.3% citric acid (pH 3.6), pure orange juice, and Sprite on creating erosive lesions on human dental enamel.

Study-3: To examine a modified cycling technique when comparing the effect of three different concentrations of fluoride toothpastes (0 ppm F, 1100 ppm F and 1384 ppm F) on human dental enamel erosion caused by application of 0.3% citric acid (pH 3.6) at room temperature.

2.4.1 Enamel and Dentine Slab Preparation

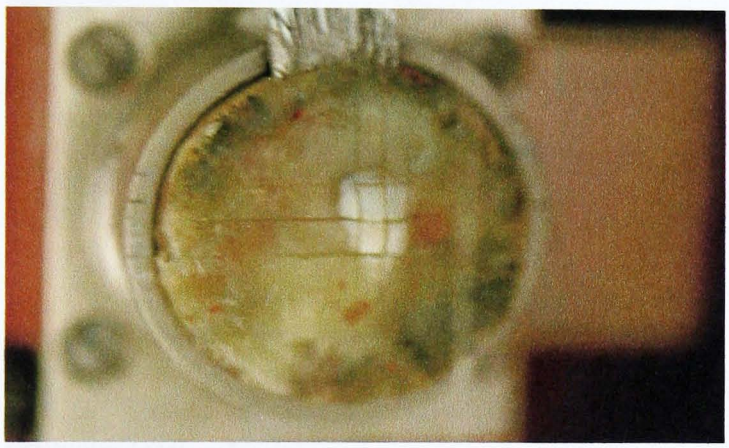
The slabs that were used in the study were from human premolars extracted for orthodontic reasons and stored in a solution of distilled water and 0.1% thymol (Sigma Aldrich). Before sectioning, the teeth were cleaned using a spoon excavator and a toothbrush with pumice powder and stone to remove any soft tissue. The crowns were carefully checked for cracks, caries, or other malformations by transillumination and reflected light low power microscopy (Leitz, Wetzlar®, Germany). Then the crowns were polished whilst wet using fine grit abrasive paper (wet or dry paper, 3M) 1200 grade 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 roots were polished whilst wet using fine grit abrasive paper (wet or dry paper, 3M) 600 and 1200 grade respectively to expose dentine by removing the cementum layer.

Two different groups of slabs were needed for the first study (enamel and dentine). The first group of slabs were examined for surface hardness and calcium and phosphate ratios (Ca:P). This was assessed by means of KMH. Surface profile and surface permeability were assessed using SP and CLSM respectively. The second group of slabs were used for this purpose.

A Well Diamond Wire Saw, water-cooled, cutting machine for sectioning was used (Well® Walter EBNER, CH-2400 Le Loche) (Figure 2.4 and Figure 2.5).

Figure 2.4: Yellowstick impression disc (Kerr) with a tooth fitted onto it. The buccal surface of the premolar was separated. The thickness of the separated part was about 2 mm.

Figure 2.5: Enamel after sectioning for four slabs to be used for Microhardness test.



For the second and third studies on enamel and dentine, three slabs from each tooth were sectioned and were assigned to the three different products used for each study (study-2 : 0.3% citric acid and pH 3.6, pure orange juice, and sprite; or study-3: 0 ppm F, 1100 ppm F and 1384 ppm F).

Then, the slabs were mounted in circular resin blocks of 3 mm thickness and 7.5 mm width to ensure flatness of their surfaces. This was achieved using a rectangular steel block which has a circular hole of 3 mm depth. Fine grit abrasive paper 600 grade (Wet or Dry paper, 3M) followed by 1200 and 2500 grade were used respectively to grind enamel surfaces after mounting in resin to the same thickness as the hole in the steel block. The slabs were then cleaned with methanol to remove any remnants of abrasive paper. Surfaces were then polished with 5 μm and 1 μm alumina paste. Thereafter, these slabs were cleaned with de-ionised distilled water and methanol and then covered with

nail varnish (red colour, MaxFactor®, England, UK) except for a small window that was left exposed (Figure 2.6 and

Figure 2.7).

Figure 2.6: Illustration shows the procedure for preparing slabs for studies 2 & 3.

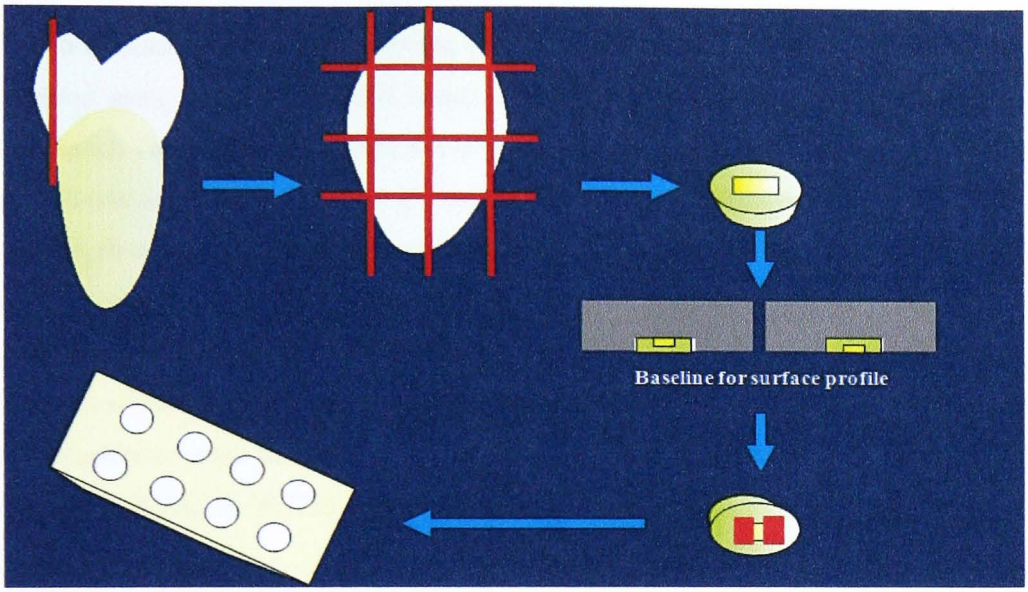
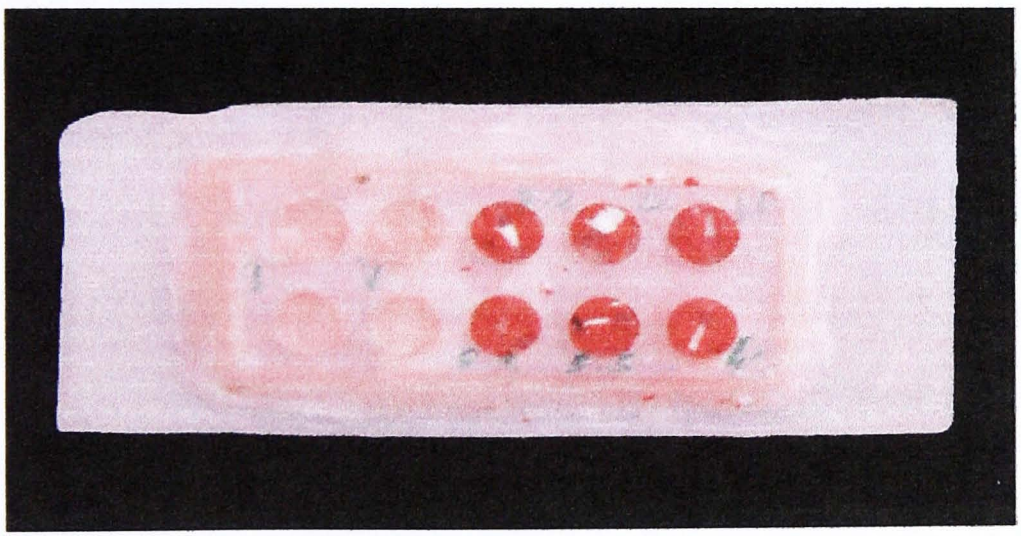


Figure 2.7: The slabs within resin blocks and held in a special holder created to hold each test group used in this part.



2.4.2 Storage of Enamel Slabs

Once the slabs had been prepared, they were kept moist in de-ionised distilled water in micro-centrifuge tubes and left at room temperature. This process was repeated after finishing the experiment.

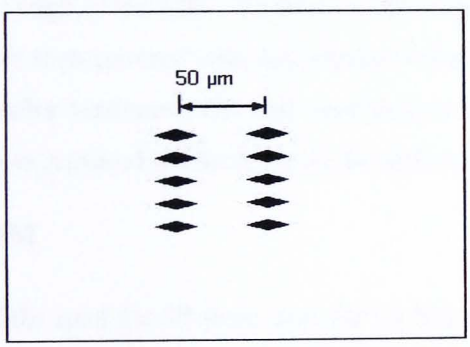
2.5 Test Methods

The following methods were used in study-1 for enamel and dentine.

2.5.1 KMH

Baseline measurements were recorded using Knoop microhardness. Microhardness was assessed using a computer-aided Duramin Indenter Machine (Struers A/S, DK 26-10, Denmark). The indentations were made using a Knoop diamond under a 100 g load for 30 seconds for enamel and a 50 g load for 30 seconds for dentine (Zero *et al.*, 1990). The length of indenter penetration was measured by means of an image analysis system. Five indentations, spaced 50 µm apart (Figure 2.8), were made for each slab and the mean was determined. The length of each indent was recorded three times and the mean was calculated. The same procedure was followed at the end of this phase. Microhardness was assessed using a computer-aided Duramin Indenter Machine (Struers A/S, DK 26-10, Denmark).

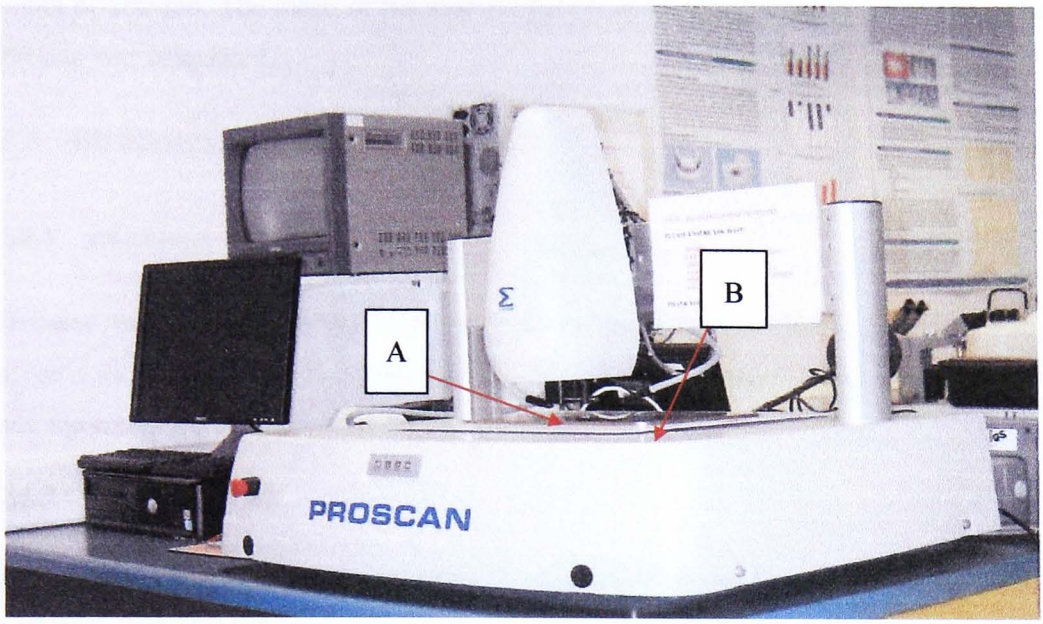
Figure 2.8: Diagram showing the gap between KMH indents



2.5.2 Surface Profilometry

Baseline measurements of the surface profile of the slabs were assessed using a surface profilometer (Scantron ProScan 2000) (Figure 2.9) to ensure that the average height to the average depth range was ± 1.0 µm.

Figure 2.9: Figure shows the surface profilometry (Scantron ProScan-2000) with its components. A: Sensor, B: Key stage.



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 used was 0.01 mm. After scanning, the average height to the average depth range (Rz) of five lines (2D) was measured. The lines were located at 0.1 cm, 0.4 cm, 0.7 cm, 1.0 cm, 1.4 cm from the edge of the slab. The mean of those lines was calculated. The enamel slabs' surfaces were then covered with nail varnish except for a small window in the middle of each slab. After treatment, the nail varnish was removed using acetone and the same procedure was repeated to check the depth surface loss (SL).

2.5.3 CLSM

The same slabs used for SP were used for CLSM to examine the porosity of the enamel or dentine surface. The enamel or dentine slabs were dipped in an aqueous solution of 0.01% fluorescein for 3 minutes (Shren *et al.*, 1990). The permeability of the enamel or dentine surface was examined by measuring the intensity of fluorescein in the eroded area compared with the intensity of fluorescein in the intact area. A Leica Laser Scanning Confocal Microscope and associated software were used for this purpose (Leica Confocal Software, Leica Microsystems, Germany). All analyses were performed under the same operating conditions of magnification (5x), gain (500-600 v), offset (-30.0 to -20.0%), and pinhole (425.00 µm). The statistical intensity values within the region of interests (eroded lesion and intact surface) were calculated using the

prescribed software. Fluorescein intensity was measured every 5 μm up to a standard depth of 100 μm. The mean of the scan measured in the eroded lesion and in the intact surface was calculated.

2.6 Blindness and Randomisation

2.6.1 Blindness

Because both toothpastes and erosive products were distinguishable, the slabs were given a new code prior to measurement. The treatment measurements were recorded and the codes were broken afterwards.

2.6.2 Randomisation

Enamel slabs were randomly allocated to each study group. Distribution of the slabs into groups was based on whether their assigned number was odd or even.

2.7 Validity

All four methods were validated with another examiner. Validity measurements were performed on dentine and enamel. Observations were used to assess validity (Appendix 1 to Appendix 6).

2.8 Experimental Protocol/Regime

2.8.1 Study-1:

A special tray with 8 holes that fitted the resin blocks was used to hold the blocks (

Figure 2.7). Resin blocks were secured in position using adhesive wax. The slabs were immersed in a static condition for two minutes five times daily in 0.3% citric acid (pH 3.6) for a period of one week. The total exposure time was 70 minutes. Citric acid was prepared by adding three grams of mono-hydrate citric acid to one litre of de-ionised distilled water. The pH was 2.65±0.05 and then NaOH was added slowly and the pH monitored using a pH electrode (VWR international Orion, Orion research, UK) during the process until the pH reached 3.60 at room temperature. Each group of slabs (8 slabs) was immersed at room temperature in fresh 200 ml aliquots of citric acid each time. On each occasion, before immersion in citric acid, the slabs were taken out of the artificial

saliva and rinsed with de-ionised distilled water (pH 6.85±0.05). The slabs were also rinsed in de-ionised distilled water after treatment before they were returned to the artificial saliva which was changed daily. The artificial saliva (Almståhl and Wikström, 2003) used had the following composition (pH 6.8):

1. NaCl (4 mM).
2. $NaHCO_3$ (14 mM).
3. $CaCl_2.H_2O$ (0.48 mM).
4. KH_2PO_4 (2.8 mM).
5. KCl (17.2 mM).

Between immersions in citric acid the slabs were left immersed in artificial saliva for 60 minutes to enable remineralisation. The slabs were kept in an incubator at 37.0°C at all times except while they were being immersed in citric acid.

At the end of the cycling period, the slabs were rinsed with de-ionised distilled water and air dried. The nail varnish was then removed using acetone and the enamel surface was cleaned with ethanol to ensure that all residues were removed. The slabs were then kept moist in de-ionised distilled water in micro-centrifuge tubes and left at room temperature.

The codes for the slabs were randomly changed after treatment to keep the study blind. The measurements were repeated five times to check the reproducibility of the methods and to determine the standard deviations when assessing the sensitivity of the methods for detecting changes caused by the erosive challenge.

2.8.2 Study-2 (Appendix-7):

A special tray with 8 holes that fitted the resin blocks was used to hold the blocks. Resin blocks were secured in position with adhesive wax. The slabs were immersed in a static condition for 2 minutes five times daily in 0.3% citric acid (pH 3.6), pure orange juice from concentrate pH 3.85 (DBC food service, Herts, UK), and Sprite pH 3.05 (Coca Cola enterprise Ltd., Uxbridge, UK) for a period of one week. The total exposure time was 70 minutes.

The immersion in erosive products was performed at room temperature. Each group of slabs was immersed in fresh 200 ml aliquots of erosive products each time. On each occasion, before immersion the slabs were taken out of the artificial saliva and rinsed

with de-ionised distilled water (pH 6.85±0.05). The slabs were also rinsed in de-ionised distilled water after treatment before they were returned to the artificial saliva. The slabs were left in a static condition during dipping in the erosive products and rinsed in de-ionised distilled water after treatment. The slabs were immersed in artificial saliva for 60 minutes to enable remineralisation. The artificial saliva was changed daily.

The erosive products were left at room temperature during the experiment. Fresh orange juice and Sprite were used daily. After treatment, the slabs were rinsed with de-ionised distilled water and air dried. Then, nail varnish was removed using acetone and the enamel surface was cleaned with ethanol to ensure that all residues were removed. The codes of the slabs were randomly changed after treatment to keep the study blind.

2.8.3 Study-3 (Appendix-8):

A special tray with 8 holes that fitted the resin blocks was used to hold the blocks. Resin blocks were secured in position using adhesive wax. The slabs were immersed in a static condition for 2 minutes five times daily in 0.3% citric acid (pH 3.6) making a total exposure time of 70 minutes. In addition, the slabs were brushed twice daily, once before the cycling with citric acid and the other after cycling with citric acid. Three groups of slabs were used for the three brushing toothpastes. The three toothpastes used were 0 ppm F, 1100 ppm F, and 1384 ppm F. Three minutes was used as an average time of brushing. Although the force applied during brushing was not measured the same person (the study investigator) carried out all brushing by hand. The slabs were placed in a special tray made to hold them and left on the bench during brushing. All slabs in each group were brushed at the same time. ASDA economic (medium) toothbrushes were used for brushing. The intervals between brushing and dipping in citric acid were 90 minutes. Three grams of toothpaste was added to 10 ml of de-ionised distilled water and mixed using a stirrer.

The immersion in citric acid was performed at room temperature. Each group of slabs was immersed in fresh 200 ml aliquots of erosive products. On each occasion, before immersion in the erosive challenge, the slabs were taken out of the artificial saliva and rinsed with de-ionised distilled water (pH 6.85±0.05). After brushing with toothpaste or immersing in citric acid, the slabs were rinsed with de-ionised distilled water. Between immersions in citric acid, the slabs were left immersed in artificial saliva for 60 minutes

for remineralisation. The enamel slabs were stored overnight in artificial saliva between acid treatments (Figure 2.10). The artificial saliva was changed daily.

After cycling, the slabs were rinsed with de-ionised distilled water and air dried. The nail varnish was then removed using acetone and the enamel surface was cleaned with ethanol to ensure that all residues were removed.

The codes of the enamel slabs were randomly changed after treatment to keep the study blind.

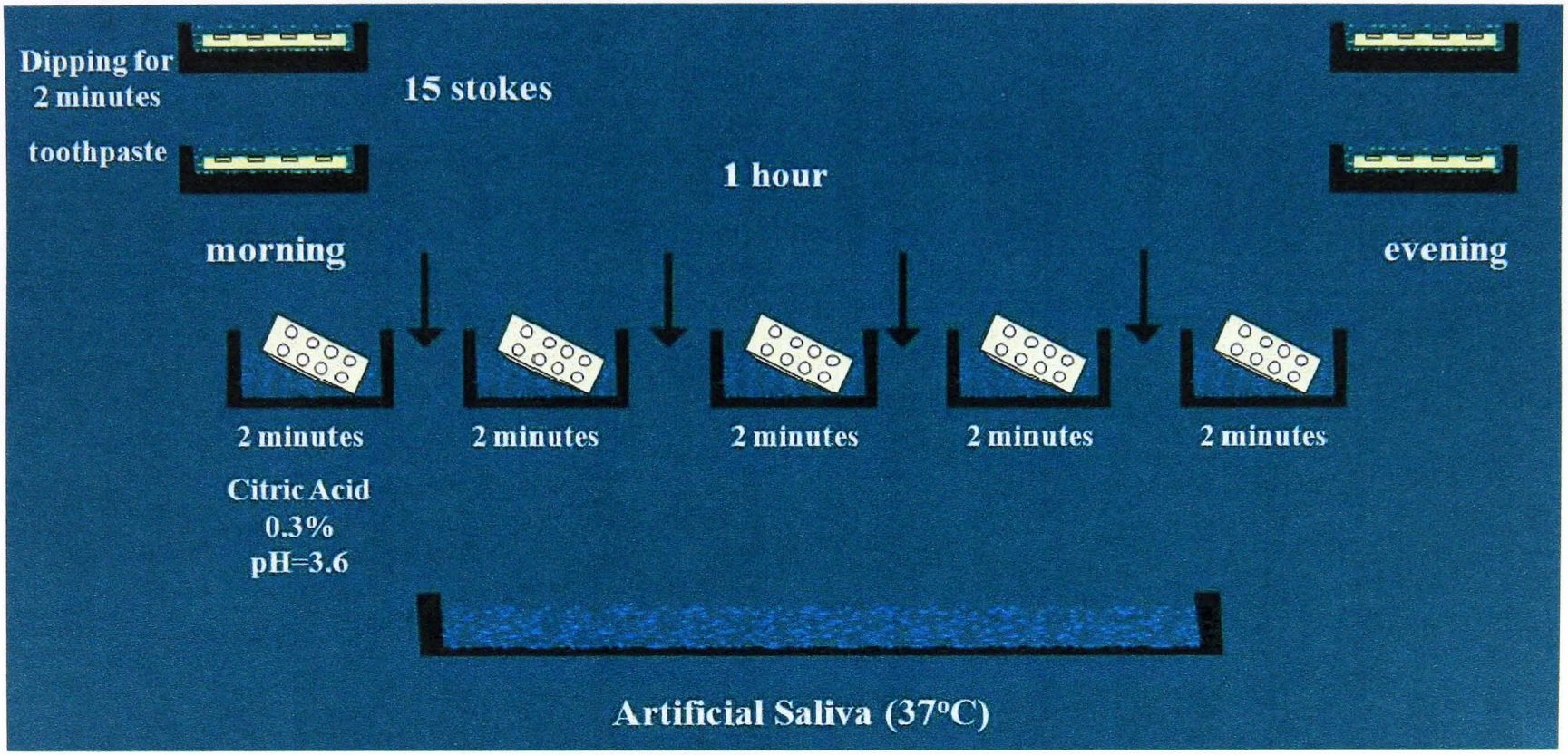
2.9 Statistics

SPSS statistical software package for Windows, version 13.0 (SPSS Inc., 1989-2002; LEAD technologies Inc., 1991-2000) was used for data analysis, and measuring the "p" values. A significance level of $p \leq 0.05$ was accepted.

For study-1, the standard deviations of the treatment measurements and the confidence interval of the changes from baselines were used to check the sensitivity of the methods in detecting the changes caused by the erosive challenge.

For studies 2 & 3, confidence intervals were used to compare the changes from baseline and or between test and/or control groups. This was because of the small sample size. Sample size was estimated for this pilot studies.

Figure 2.10: Illustration shows the cycling technique developed for this section. This technique was used later in the other studies. This cycling regime was modified each time by removing/adding one of its components (i.e dipping/brushing) depending on the aim of a particular study.



2.10 Results

This section presents the results of the study. The presentation of the results is in the same order as described in the Materials and Methods section, in order to facilitate reading. The statistical approach for the analysis of the results was expressed using confidence intervals, based on paired-sample t-test using SPSS software (version 13.0, SPSS Inc., 1989-2002; LEAD technologies Inc., 1991-2000). Appendix 9 to Appendix 22 show the detailed results.

2.10.1 Enamel results

2.10.1.1 Study-1

The mean of all the slabs for each of the test methods, the percentage SD to the mean treatment of the Knoop microhardness measurements was least for the calcium phosphate ratios (7.5%). This was followed by surface hardness (15.4%), surface loss (23.4%) and surface porosity (43.6%) as shown in Table 2.1.

Table 2.1: Comparison of the standard deviation of the treatment measurement of the four different methods

		KMH (µm)	% SD to the mean	SL (µm)	% SD to the mean	Fluorescein Intensity	% SD to the mean
N	Valid	8		8		8	
	Missing	0		0		1	
	Mean	119.6		9.7		159.1	
	SD	18.4	15.4	2.3	23.4	69.3	43.6

Two slabs were lost during the laboratory preparation process while performing the measurements for CLSM (one for each). Therefore the sample size was 8 for KMH and SP, and 7 for CLSM. The following table shows the standard deviation (SD) of the 5 treatment measurements of all the methods performed on each individual slab and the percentage of the SD to the mean treatment for the five measurements (Table 2.2):

Table 2.2: Standard deviation (SD) of the five measurements of all the methods for each individual slabs and the percentage of the SD to the mean of the five measurements.

		KMH (µm)	% SD to the mean	SL (µm)	% SD to the mean	Fluorescein Intensity	% SD to the mean
N	Valid	5		5			5
	Missing	0		0			0
Slab-1	Mean	155.4		9.8		50.5	
	SD	7.1	4.6	0.7	7.4	4.2	8.3
Slab-2	Mean	105.3		7.3		103.7	
	SD	5.2	5.0	0.2	3.3	10.8	10.5
Slab-3	Mean	116.5		14.4		253.8	
	SD	6.7	5.8	1.7	12.0	0.2	0.1
Slab-4	Mean	131.6		9.4		176.5	
	SD	5.8	4.4	0.3	3.5	44.7	25.3
Slab-5	Mean	114.9		9.5		172.1	
	SD	3.2	2.8	0.5	5.0	22.9	13.3
Slab-6	Mean	100.2		8.1		221.8	
	SD	10.6	0.6	2.3	28.9	1.5	0.7
Slab-7	Mean	104.6		9.9		135.2	
	SD	5.0	4.8	1.1	11.2	47.0	34.8
Slab-8	Mean	127.9		9.2			
	SD	9.8	7.6	0.7	7.6		

The mean change and the standard deviation (SD) of each methodology in this study were used to calculate a percentage of SD to the mean (% SD to the mean). This was agreed statisitically to measure the reliability of each method to detect changes occurred during experiment. The above table shows that the percentage of the SD to the mean treatment of the Knoop microhardness measurements ranged from 0.6% to 10.6% and for surface loss or fluorescein intensity ranged from 3.5% to 28.9% or 0.7% to 34.8% respectively. The changes from baseline were significant for all the methods as assessed from the confidence intervals (Table 2.3).

Table 2.3: Comparison of the change from baseline for the four different methods. Negative values mean that there is a change from baseline in all methods.

	Mean	SD	SE	95% Confidence Interval of the Difference	
				Lower	Upper
Confocal	-129.08	67.22	25.41	-191.24	-66.91
Microhardness	-54.99	18.26	6.46	-70.26	-39.72
Profilometer	-8.44	2.13	.75	-10.23	-6.66

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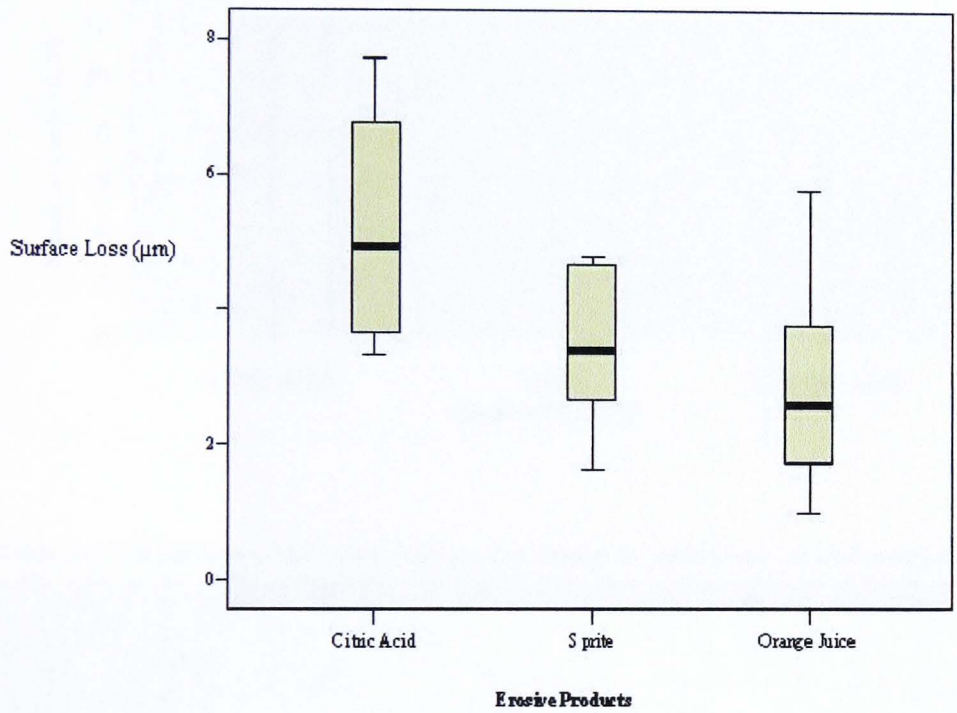
2.10.1.2 Study-2

Surface loss (SL) measured with surface profilometer scanning (Scantron ProScan) was used to evaluate the changes of the eroded enamel lesions.

All the erosive products (citric acid, orange juice, and Sprite) showed changes from baseline (Figure 2.11).

Figure 2.11: Median change of surface loss from baseline caused by the three erosive products.



However, citric acid showed the largest change in surface loss in enamel. This was significant ($p \leq 0.05$) when compared with orange juice and Sprite using confidence intervals. However, there was no significant change between Sprite and orange juice (Figure 2.12 and

Table 2.4).

Figure 2.12: Mean change in surface loss (enamel wear) caused by 0.3% citric acid (pH 3.6), orange juice and Sprite on creation of erosive lesions of dental enamel. Error bars represents the confidence intervals (95%).

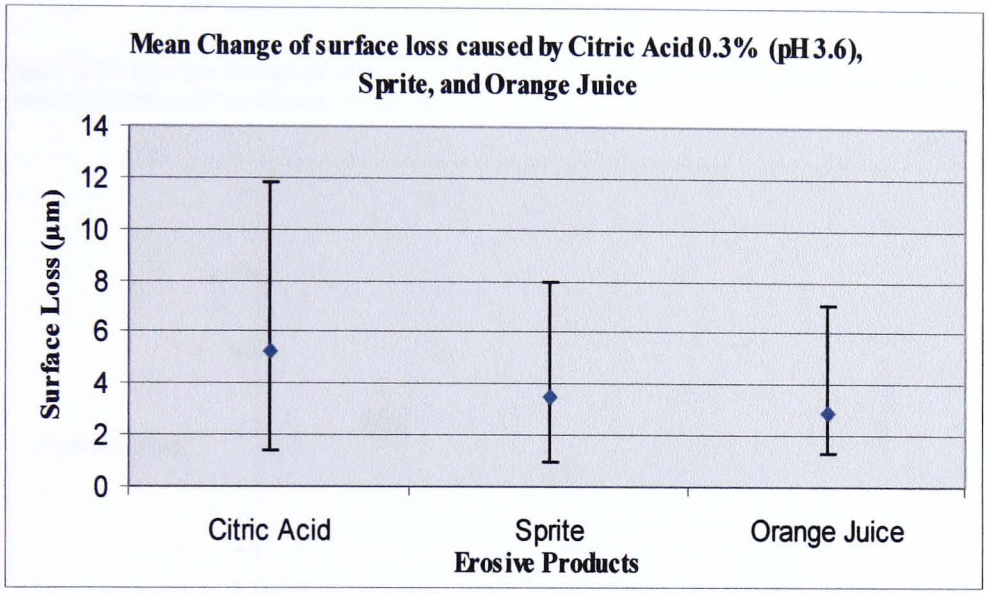


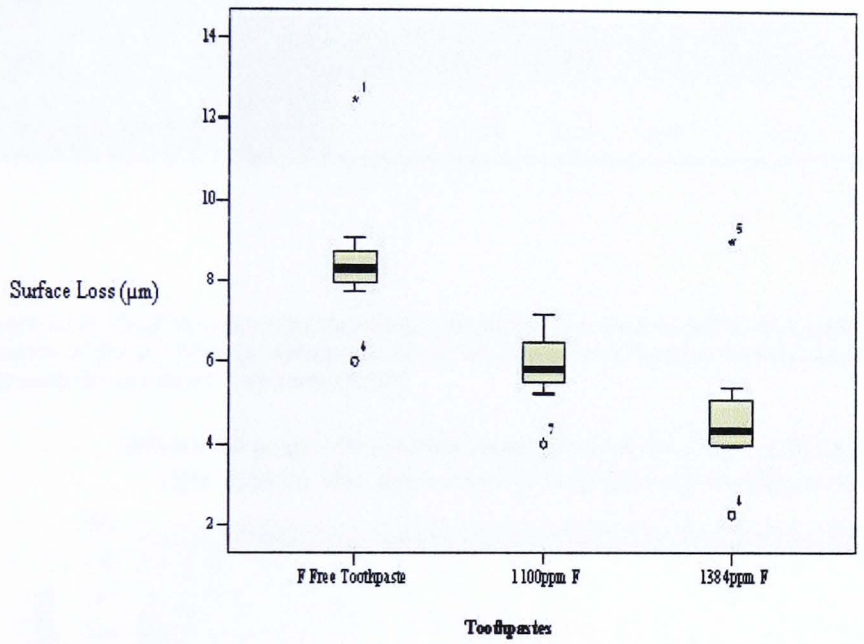
Table 2.4: Comparison of the mean difference of change in surface loss (enamel wear) caused by 0.3% citric acid (pH3.6), orange juice and Sprite on creation of erosive lesions of dental enamel.

			n	Mean	SD	SE	95% Confidence Interval of the Difference	
							Lower	Upper
Citric Acid	**VS**	**Sprite**	8	1.73	1.11	0.39	0.80	2.66
Citric Acid	**VS**	**Orange Juice**	8	2.35	1.58	0.56	1.03	3.67
Sprite	**VS**	**Orange Juice**	8	0.62	1.22	0.43	-0.40	1.64

2.10.1.3 Study-3

The three toothpastes showed changes from baseline (Figure 2.13).

Figure 2.13: Median change of surface loss from baseline caused by three erosive products. We can notice the outliers of each group (° and *)

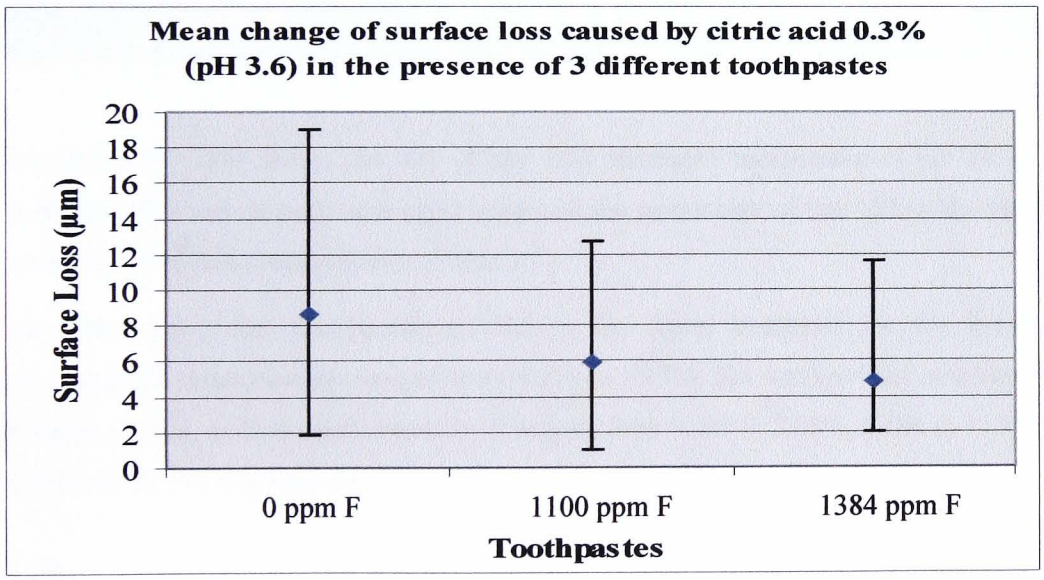


Less surface loss was observed with either of the fluoridated toothpastes compared to the non-fluoridated toothpaste. This difference was significant when confidence interval were compared. There was no significant difference between toothpastes containing fluoride. However, the toothpaste containing 1384 ppm F was associated with less surface loss than the toothpaste containing 1100 ppm fluoride (Table 2.5 and Figure 2.14).

Table 2.5: Comparison of the mean change in surface loss (enamel wear) caused by 0.3% citric acid (pH 3.6) in the presence of 3 different concentrations of fluoride toothpastes on creation of erosive lesions of dental enamel.

			N	Mean	SD	SE	95% Confidence Interval of the Difference	
							Lower	Upper
0 ppm F	**VS**	**1100 ppm F**	7	2.72	1.77	0.67	1.08	4.36
0 ppm F	**VS**	**1384 ppm F**	7	3.77	2.46	0.93	1.49	6.04
1100 ppm F	**VS**	**1384 ppm F**	7	1.04	2.35	0.88	-1.13	3.22

Figure 2.14: Mean changes in surface loss (enamel wear) caused by 0.3% citric acid (pH 3.6), in the presence of three different toothpastes on creation of erosive lesions of dental enamel. Error bars represent the confidence intervals (95%).



2.10.2 Dentine results

2.10.2.1 Study-1

The percentage SD to the mean treatment results for the Knoop microhardness measurements was least for the Ca:P ratios (9.5%). This was followed by surface hardness (11.7%), surface porosity (34.3%) and surface loss (35.9%) as shown in Table 2.6

Table 2.6: Comparison of the SD of the treatment measurement of the four different methods on dentine

		KMH (µm)	% SD to the mean	SL (µm)	% SD to the mean	Fluorocien Intensity	% SD to the mean
N	Valid	7		7		7	
	Missing	0		0		0	
	Mean	113.3		3.33		92.6	
	SD	13.3	11.7	1.19	35.9	31.7	34.3

The following table shows the SD of the five treatment measurements for all the methods performed on each individual slabs and the percentage of that SD to the mean treatment of the five measurements (Table 2.7).

The table shows that the percentage SD to the mean treatment for the Knoop microhardness measurements ranged from 6.3% to 15.3%. For surface loss, calcium to phosphate ratios, or fluorescein intensity it ranged from 6.0% to 24.8%, 1.0% to 11.8%, or 3.8% to 10.5% respectively.

Table 2.7: SD of the five measurements of all methods on each individual slab and the % SD to the mean of the five measurements on dentine.

		KMH (µm)	% SD to the mean	SL (µm)	% SD to the mean	Fluorocien Intensity	% SD to the mean
N	Valid	5	5	5	5	5	5
	Missing	0	0	0	0	0	0
Slab-1	Mean	109.7		5.67		71.7	
	SD	7.6	7.0	0.62	10.92	4.1	5.7
Slab-2	Mean	124.8		2.30		103.7	
	SD	11.7	9.4	0.57	24.80	10.8	10.5
Slab-3	Mean	112.3		3.15		150.7	
	SD	7.1	6.3	0.40	12.60	5.8	3.8
Slab-4	Mean	100.6		2.19		52.4	
	SD	12.3	12.2	0.33	15.02	5.2	9.9
Slab-5	Mean	125.1		2.74		114.4	
	SD	19.1	15.3	0.17	6.05	6.5	5.7
Slab-6	Mean	107.0		3.87		93.9	
	SD	7.8	7.3	0.47	12.07	5.4	5.7
Slab-7	Mean	113.5		3.36		66.4	
	SD	8.8	7.8	0.52	15.47	3.1	4.7

Again, the mean change and the standard deviation (SD) of each methodology in this study were used to calculate a percentage of SD to the mean (% SD to the mean). This was agreed statisitically to measure the reliability of each method to detect changes occurred during experiment. The changes from baseline showed significant difference for all methods. This was determined by comparing between the confidence intervals of all the techniques (Table 2.8).

Table 2.8: Comparison of the change from baseline for the four different methods on dentine.

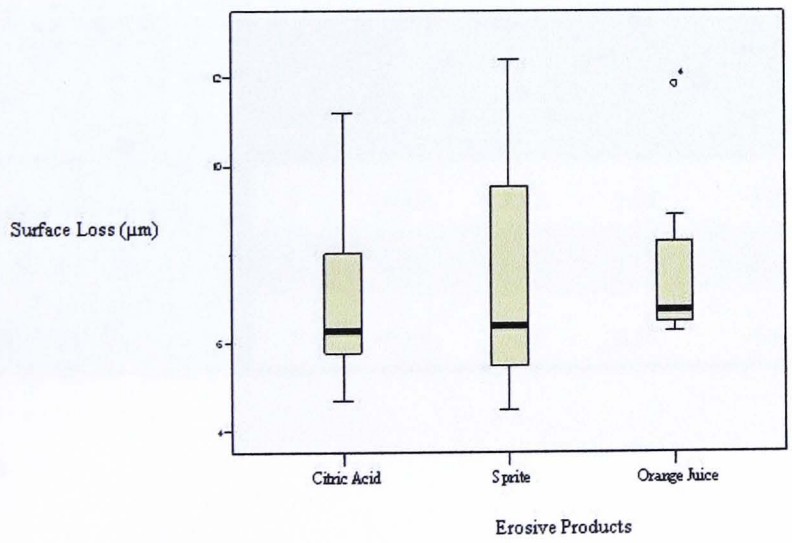
	n	Mean	SD	SE	95% Confidence Interval of the Difference	
					Lower	Upper
Confocal	7	-91.0	34.5	13.1	-122.9	-59.0
Microhardness	7	-26.4	3.9	1.47	-30.0	-22.8
Profilometer	7	-2.8	1.1	0.4	-3.8	-1.7

2.10.2.2 Study-2

Surface loss with a scanning surface profilometer (Scantron ProScan) was used to evaluate the changes of the eroded dentine lesions.

All erosive products (citric acid, orange juice, and Sprite) showed changes from baseline (Figure 2.15).

Figure 2.15: Median change of surface loss from baseline caused by three erosive products.



However, there was no significant difference between erosive products when the confidence intervals of the paired t-test were compared (Figure 2.16 and Table 2.9).

Figure 2.16: Mean changes in surface loss (enamel wear) caused by 0.3% citric acid (pH 3.6), orange juice and Sprite on creation of erosive lesions on dentine. Error bars represent the confidence intervals (OJ=Orange Juice).

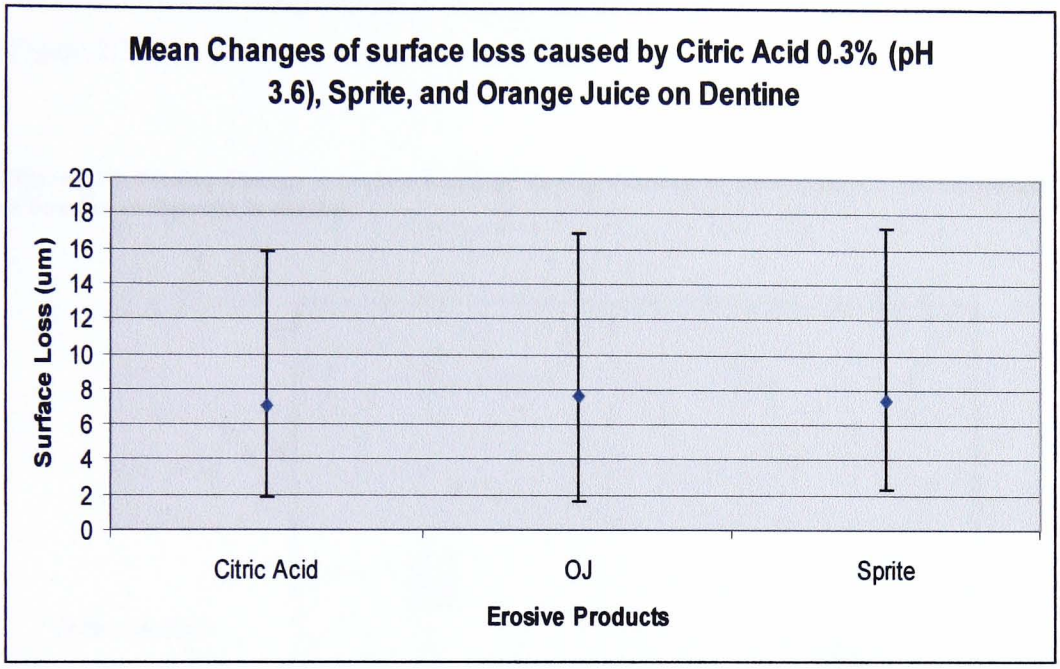


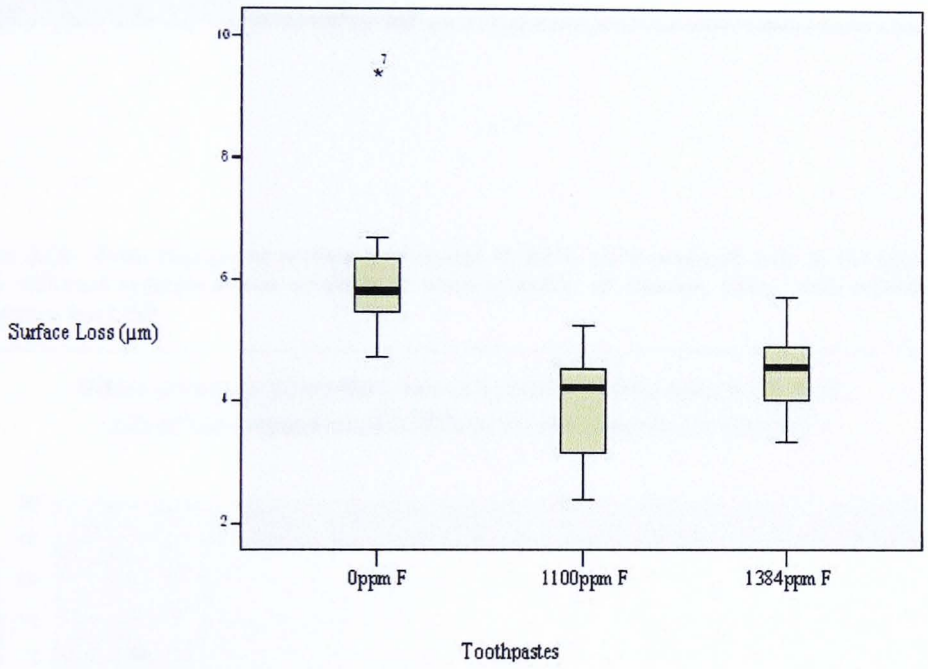
Table 2.9: Comparison of the mean changes in surface loss (enamel wear) caused by 0.3% citric acid (pH 3.6), orange juice and Sprite on creation of erosive lesions on dentine.

	n	Mean	SD	SE	95% Confidence Interval of the Difference	
					Lower	Upper
Citric Acid VS Sprite	7	-0.45	3.33	1.18	-3.23	2.34
Citric Acid VS Orange Juice	7	-0.63	3.31	1.17	-3.40	2.13
Sprite VS Orange Juice	7	-0.19	1.55	0.55	-1.48	1.11

2.10.2.3 Study-3

The three different concentrations of fluoride toothpastes showed changes from baseline (Figure 2.17).

Figure 2.17: Median Change of surface loss from base line caused by three different concentrations of fluoride toothpastes in dentine.

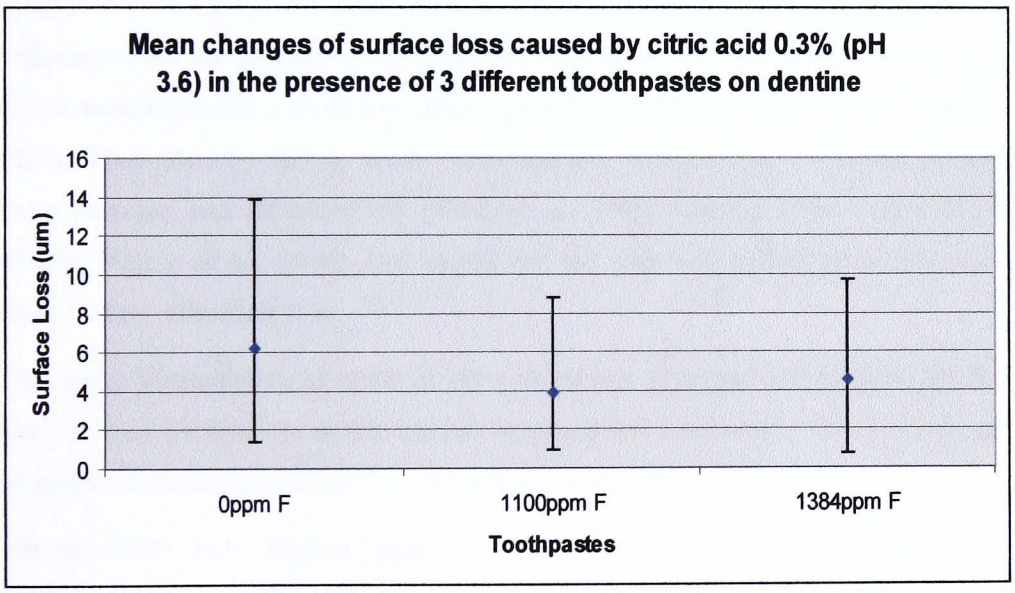


Both fluoridated toothpastes were associated with less surface loss of dentine compared to the non–fluoridated toothpaste. This difference was significant when confidence intervals were compared. However, there was no significant difference between the fluoridated toothpastes. The toothpaste containing 1384 ppm F was associated with less surface loss than the toothpaste containing 1100 ppm F (Table 2.10 and Figure 2.18).

Table 2.10: Comparison of the mean changes in surface loss caused by 0.3% citric acid (pH 3.6) in the presence of three different toothpastes on creation of erosive lesion on dentine.

	N	Mean	SD	SE	95% Confidence Interval of the Difference	
					Lower	Upper
0 ppm F VS 1100 ppm F	7	2.31	1.58	0.60	0.85	3.78
0 ppm VS 1384 ppm F	7	1.71	1.47	0.55	0.35	3.06
1100 ppm F VS 1384 ppm F	7	-0.61	1.39	0.53	-1.89	0.68

Figure 2.18: Mean changes in surface loss caused by 0.3% citric acid (pH 3.6), in the presence of three different toothpastes on creation of erosive lesions of dentine. Error bars represent the confidence intervals.



2.11 Discussion

In this section, the findings of the outcomes of the four methods used to assess erosion are discussed. Moreover, the potential erosive effects of 0.3% citric acid (pH 3.6), orange juice and Sprite used in this study are discussed. In addition, the effect of three different concentrations of fluoride toothpastes on the eroded dental lesions under erosive challenge (0.3% citric acid, pH 3.6) is discussed.

2.11.1 Study Design

In vitro models of various designs have been used for the evaluation of tooth surface loss due to the interaction of erosion and abrasion. For simulating intra-oral erosion it is desirable to assess the effects on native tooth surfaces, however for the precise assessment of tooth surface loss or for creating a reference surface for the *in vitro* studies, the outer surface needs to be removed (Ganss *et al.*, 2000).

Studies *in vitro* offer a well controlled environment (Hall *et al.*, 1998) but they lack any influence from the numerous variables encountered in the oral environment, most of which would provide a protective effect against acid erosion such as rapid clearance of the acid substrate by saliva, saliva flow rate and composition, formation of pellicle, tooth anatomy and structure, etc (West *et al.*, 1998; Hannig, 1999; Amaechi *et al.*, 1999b; Wetton *et al.*, 2006). Our model did not take into consideration the effect of these factors on surface loss.

The design attempted to simulate *in vitro* commonly practised oral hygiene habits. We used an erosion/abrasion model that has been applied successfully for the investigation of enamel and dentine surface loss but still needs further evaluation.

The six times daily dipping regime in an erosive challenge for 5 minutes on each occasion has been used in previous *in vitro* studies (Amaechi *et al.*, 1998a and 1999c). However, this is probably an over-estimation of the "real life" situation. Therefore, we used a five times daily dipping regime under erosive challenge for two minutes on each occasion.

2.11.2 Data Handling and Statistics

The present studies were pilot studies to make an initial evaluation of the method, erosive challenges, and fluoride effect on dental erosion prior to carrying out a more extensive research project. In view of this the results were interpreted in terms of confidence intervals and standard deviations. The sample size was estimated for these studies considering them as pilot studies.

2.11.3 Evaluation Techniques

2.11.3.1 Microhardness testing

Microhardness testing is a sensitive method to assess enamel de- or remineralisation (Zero *et al.*, 1992). Feagin *et al.* (1969) measured the calcium and phosphate loss or gain during enamel remineralisation or softening and showed that the values correlated well with the enamel mirohardness testing values. Similar results have been found for etched enamel (Davidson *et al.*, 1974) and therefore microhardness testing has also been used to assess surface loss due to erosion or erosion/abrasion (Jaeggi and Lussi, 1999).

The advantage of this technique is that it permits sequential measurements to be undertaken during the study quickly and simply. It provides however, an indirect measurement of enamel loss or gain as opposed to other direct techniques such as TMR (ten Bosch and Angmar-Mansson, 1991; Featherstone, 1992).

Various loads of the Knoop diamond have been applied in the plethora of cariogenicity studies ranging from 50g up to 500g and it is recommended that a load between 50-200g be used (Featherstone, 1992). It has been shown (Graig and Peyton, 1958) that a 50g load results in well-defined indentations with a minimum of fractures around the edges. However, it was observed by Davidson *et al.* (1974) that a 100 g load was necessary in order to facilitate optical perceptibility. In this study a 100 g load was chosen for the above reason.

Reproducibility and reliability were satisfactory in this study. Although variation in microhardness values can be observed even between different sites of the same tooth due to the differences in fluoride intake across the enamel surface (Caldwell *et al.*, 1957) it was considered more appropriate to standardise the origin of the slabs in order

to eliminate any errors due to natural biological variation in the specimens. Therefore in the future work, surface microhardness was used as an inclusion criterion. .

2.11.3.2 Profilometry

Profilometry has been one of the most common lab techniques for assessing tooth surface loss. It provides an accurate, highly reproducible, simple and fast assessment over a relatively large area of enamel. However, the samples have to be ground flat prior to use (Barbour and Rees, 2004).

The optical profilometer implemented in this study has got the advantage that no direct physical contact with the assessed surface is applied and no damage occurs to the surface by scratching of the soft eroded/abraded surface. It provides accurate measurements even when the specimen is tilted as the surface can be levelled horizontally and therefore the position of the slab is not critical for the accuracy and reproducibility of the measurements. It scans the whole surface of the sample within less than a minute and the data are small enough (in computer capacity) to be processed quickly.

Surface profilometry has been used to assess the erosive potential of various products *in vitro* including herbal teas (Phelan and Rees, 2003), various acid solutions (Hughes *et al.*, 2000) toothpastes and CPP-ACP products (Rees *et al.*, 2007) and mouthrinses (Pontefract *et al.*, 2001). The technique has also been adapted for use in clinical trials.

It is a fast and easy method to evaluate surface loss with high precision provided that the tooth loss exceeds about 0.4 μm (Hooper *et al.*, 2003). A meticulous flattening and polishing of the tooth surface is important in order to accomplish reliable detection of minimal loss even below 1 μm (Barbour and Rees, 2004). In the present study, the main problem we had to overcome with the surface profilometer test was to achieve flat and reproducible surfaces. This step of the study has been proven time consuming and we needed to repeat grinding 2-3 times for some slabs. However care was taken to preserve the thickness of the slabs.

2.11.3.3 Confocal laser scanning microscopy

Confocal laser scanning microscopy (CLSM) has been applied in dental research relatively recently. Its use has been confined in quantitative determination of the surface

roughness of dental materials and in qualitative comparison of the surface characteristics after different treatments. It has also been shown that confocal microscopic examination of enamel specimens can detect carious lesions in earlier stages than microradiography (Sønju Clasen *et al.*, 1997).

In erosion/abrasion studies confocal laser scanning microscopy has been used either as an adjunct method for qualitative assessment of the surface or as a quantitative method in evaluation of flat surfaces (Kodaka *et al.*, 1993; Kodaka *et al.*, 1999).

As one of the advantages of the CLSM is that no special preparation of the specimens is required. However, many difficulties were encountered during this attempt. This is was particularly in enamel samples where it was not possible to measure this ratio because of the precipitation on the surface noticed.

Other difficulties which however, could not be overcome were related with resolution problems due to vibration and due to the rise of temperature in the room. Unfortunately, the CLSM was not positioned on an anti-vibration table, which compromised the resolution (Watson, 1997). Lower resolution was obtained as well due to the rise in room temperature from the laser fan. One degree of rise in temperature can result in a decrease in resolution of approximately 1μm.

In our study, we tried to measure the intensity of chlorofin absorbed by eroded enamel surface. This had two questions about this technique. The first is the actual scanning depth that can be achieved. This was not more than 100 μm depth. Therefore, it was not possible to decide if the chlorofin has penetrated to deeper layer than this. Therefore, we tried to measure the intensity of chlorofin up to 100 μm depth. This raised the other issue which relates to the size of chlorofin particles and the size of porous area created by erosive challenge. If the size of the pourous areas are smaller particularly in deeper layers, then the intake of chlorofin will not reflect the real life scenario. This was considered as one of the disadvantages of this technique.

2.11.4 Discussion of the results

Study-1 evaluated the reproducibility and sensitivity of four different methods for detecting any change after exposing dental slabs to erosive challenges.

For the enamel study, the mean %SD was least when we used KMH followed by SP and CLSM respectively. All three methods had an acceptable reproducibility.

In KMH, it was difficult to read the indents; therefore three readings for each indent were performed to decrease the standard error. This made the procedure rather time intensive. For CLSM, it was difficult to standardise the method because of the difficulty in deciding the beginning of the scanning area (enamel surface). This might explain the huge standard deviations we observed.

In the dentine study, we noticed that fluorescein was not absorbed by intact dentine when we removed the nail varnish. This might have been caused by sealing of the dentinal tubules with nail varnish.

The surface profilometer method was used in the following studies because of its sensitivity to detect the changes caused by erosive challenges and its acceptable reproducibility. However, all the methods used were user-friendly and reproducible. The equipment was also user-friendly and easy to use.

In study-2 on enamel, all erosive products (citric acid, Sprite, and orange juice) produced measurable erosive lesions *in vitro*. However, citric acid seemed to be the best for producing these lesions. In addition, citric acid has the advantage of being prepared fresh and constantly in the lab. Therefore it was used in the subsequent studies.

Similar results were observed in study-2 on dentine. However, there was no difference between erosive products. Citric acid was used in the following study due to the above mentioned reasons.

Study-3 showed that fluoride had an effect on dental erosion on both enamel and dentine. However, there was no statistical difference between the 1384 ppm F and 1100 ppm F toothpastes. This might be because the treatment duration was not long enough to show if there was any difference between the groups. It is also possible that fluoride after a certain threshold level has no further significant difference on erosion. To test this hypothesis high fluoride toothpaste would have to be compared to low fluoride toothpastes to determine any threshold dose limit for fluoride.

2.12 Conclusion

1. There were no differences in the reliability of the following methods in detecting changes on enamel hard tissues under citric acid erosive challenge: SKM, SP, and CLSM. This accepts the null hypothesis for study-1. However, the surface profilometry technique is a user friendly method to examine dental erosion with acceptable reproducibility.
2. There were no differences between citric acid (0.3% pH 3.6), pure orange juice, or Sprite in creating erosive lesions. This accepts the null hypothesis for study-2. However, citric acid can be prepared freshly in the lab and therefore it was recommended for future use.
3. There were differences between fluoride toothpastes (1100 ppm F, 1385 ppm F) and the fluoride-free toothpaste (0 ppm F) on dental erosion. This rejects the null hypothesis for study-3. 1385 ppm F toothpaste was associated with less surface loss than 1100 ppm F. However, there was no statistical difference between the 1384 ppm F and 1100ppm fluoride toothpastes. Further research will be conducted to investigate if there was a dose response effect with fluoride concentration in the toothpastes.

3 *IN VITRO* STUDIES TO INVESTIGATE THE EFFECT OF FLUORIDE ON DENTAL EROSION

3.1 Introduction

Dental erosion is defined as irreversible loss of dental hard tissue by a chemical process that does not involve bacteria (Pindborg, 1970). The erosion process involves periods of demineralisation and remineralisation that cause dissolution of the tooth surface (Eccles and Jenkins, 1974). Dissolution of mineralised tooth structure occurs upon contact with acids that are introduced into the oral cavity from intrinsic (e.g., gastroesophageal 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.*, 2003). Increased demineralisation of the surface creates two layers, surface and subsurface layers. The surface layer is totally demineralised and repair is not possible. The sub-surface layer is partially demineralised and probably remineralised (Lussi *et al.*, 2003). The interest in dental erosion has increased dramatically over the last ten years. This increased interest was because of the decrease in dental caries and the increase interest of scientists in dental erosion (Lussi *et al.*, 2004b).

Tooth wear is recognised as a major problem in both children and adults (Nunn *et al.*, 2003). 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. The variables that affect dental erosion include pH, temperature and exposure time (Amaechi *et al.*, 1999a; Eisenburger and Addy, 2001), titratable acidity and buffering (Cairns *et al.*, 2002; Larsen and Nyvad, 1999; Lussi *et al.*, 1993), salivary pellicles (Meurman and Frank, 1991b; Amaechi *et al.*, 1999b), remineralisation effect of saliva (Amaechi and Higham, 2001; Attin *et al.*, 2003; Kelly and Smith, 1988), abrasion (Amaechi *et al.*, 2003) and fluoride (Attin et *al.*, 2003).

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 (Attin *et al.*,

2003; Lussi *et al.*, 2003; Amaechi *et al.*, 2003) and different fluoride products i.e. fluoride acidified gel (Attin *et al.*, 1999; Jones *et al.*, 2002), mouth rinse (Lussi *et al.*, 2004), and toothpaste (Kelly and Smith, 1988; Eisenburger *et al.*, 2000). Therefore, it was deemed worthwhile to try mimicking the in vivo scenario as closely as possible to produce erosive lesions and develop an evaluation technique to study dental erosion progression and development of preventive agents. In addition, trying to introduce guidelines to GDPs to refer patients with dental erosion before its progress was the aim of the clinical part of this project.

3.1.1 The influence of different factors on *in vitro* enamel erosion

In the mouth the teeth are influenced by many factors (i.e. temperature, fluoride in saliva, etc). These factors affect the demineralisation and remineralisation processes, as well as tooth surface loss.

3.1.1.1 The effect of pH, temperature and exposure time on dental erosion

The pH, temperature and exposure time of erosive products has an impact on the creation of an eroded lesion *in vitro* (Amaechi *et al.*, 1999b, Eisenburger and Addy, 2001). Amaechi *et al.*, (1999b) investigated the effect of temperature and exposure time on the dental creation of eroded enamel lesions *in vitro*. They used human and bovine enamel, exposed to orange juice at different temperatures (4, 20 or 37°C) for different lengths of time (6 times daily for 5 min on each occasion for 12, 16, 20, or 24 days making a total of 6, 8, 10, or 12 h of exposure to orange juice). Lesion parameters (mineral loss and lesion depth) were quantified using microradiography. However, a significantly less mineral loss was observed at 4°C when compared with 20°C ($p \leq 0{\cdot}01$) and 37°C ($p \leq 0{\cdot}01$), and at 20°C when compared with 37°C ($p \leq 0{\cdot}01$). A similar trend was observed with the lesion depth. It was observed that mineral loss was significantly lower after 6 h of exposure compared with 8 h ($p \leq 0{\cdot}01$), 10 h ($p \leq 0{\cdot}01$) and 12 h ($p \leq 0{\cdot}01$). At 8 h there was a significantly lower mineral loss when compared with 10 h ($p \leq 0{\cdot}01$) and 12 h ($p \leq 0{\cdot}01$). Also, a significantly lower mineral loss was observed after 10 h when compared with 12 h ($p \leq 0{\cdot}01$). The same trend was observed with lesion depth. A direct relationship (positive correlation) was observed between the mineral loss and exposure time ($r=0{\cdot}98$, $p \leq 0{\cdot}05$), and between the lesion depth and exposure time ($r=0{\cdot}99$, $p \leq 0{\cdot}01$).

Eisenburger and Addy (2001) studied the effect of various pH values of citric acid and erosion time on erosion depth and subsurface demineralisation of human enamel *in vitro*. Six groups of 10 samples were eroded in 0.3% citric acid for 2 h at pH 2.54, for 2 h or 4 h at pH 3.2 and for 2, 4 or 8 h at pH 4.5 respectively. The specimens of the first group were treated for 2 h with 0.3 % citric acid at the natural pH of 2.54. Two groups of specimens were eroded with 0.3% citric acid at pH 3.2 for 2 or 4 h, respectively. The remaining three groups were exposed to 0.3% citric acid at pH 4.5 for 2, 4 or 8 h respectively. The erosion depth was measured using a profilometer. The demineralised layer was then removed by ultrasonicating the samples, with profilometric measurements taken at 5, 30, 120, 240 and 480 sec ultrasonication time. Erosion depth increased with increasing erosion time and decreasing pH of the citric acid. A significant difference for all test groups ($p \leq 0.001$) was observed. The exceptions were no significant difference between the 2 h pH 3.2 and 4 h pH 4.5 groups and between the 4 h pH 3.2 and 8 h pH 4.5 groups. Ultrasonication resulted in an increase in lesion depth in all groups.

It appears that the starting pH of erosive products has an important impact on dental erosion. Therefore, different pH start points of erosive products were investigated in this project.

3.1.1.2 The effect of titratable acidity and buffering on dental erosion

Cairns *et al.*, (2002) evaluated the erosive effects of diluting juices by measuring their acidity levels. Four popular diluting drinks were investigated in the study: Robinson's Whole Orange Drink, Robinson's Special R No Added Sugar Orange, Ribena Original Blackcurrant and Ribena ToothKind Blackcurrant. A series of dilutions of each drink were prepared using water as the diluent. Concentrations of juice prepared ranged from neat to one part juice in 100,000 parts water. The pH of each dilution was measured using the pH electrode. Twenty millilitres of each of the first 13 dilutions (neat, 1:1–10, 1:20 and 1:50) for each drink were then titrated by adding increments of 1M sodium hydroxide solution (NaOH) and measuring the pH until pH 10 was reached. The volume of NaOH solution added was then plotted against pH and the amount required to obtain pH values of 5.5 and 7.0 determined to give a measure of the titratable acidity of each sample. Predetermined dilutions of citric acid and hydrochloric acid, with similar pH values to those of the drinks, were used as positive control acidic solutions. All four

drinks were significantly buffered, with the pH changing very little with increasing dilution ratio, compared to the citric and hydrochloric acid controls. Diluting the drinks reduced their titratable acidity in proportion with the dilution ratio. Therefore, they suggested that the erosive potential of diluting juices may be reduced by the addition of considerable amounts of water.

Larsen and Nyvad (1999) compared the pH and the buffering effect of various soft drinks with their erosive effects and the solubility of apatite. In 18 soft drinks, pH and the concentrations of calcium, phosphate and fluoride were determined. The buffering effect was determined by titration with NaOH solution. Fifty-four human teeth were covered with nail varnish except for 3x4 mm windows of enamel and were exposed to 1.5 L drink for either 7 d or 24 h under constant agitation. The depth of the erosive lesions was assessed in longitudinal sections. The depth varied greatly from 3 mm eroded by the most acidic drinks and fresh orange juice to slightly affected surfaces as with most of the mineral waters. The erosion depth induced by the drinks was less with higher pH values.

Lussi *et al.*, (1993) investigated the erosive potential of beverages and foodstuffs [grapefruit juice, apple juice, orange juice, Isostar fresh, Coca Cola, lactate (0.05 mmol/L, pH 4.75), Sprite Light, salad dressing (Pikant, Migros), white wine, Perform (a sport drink), drinking whey, and yoghurt]. The pH, the amount of base required to titrate to pH 5.5 and 7.0 as well as the buffering capacity at pH 5.5 were determined. Knoop microhardness and iodide permeability with an iodide ion-specific electrode were measured before and after exposure. A window of 6x2 mm was left exposed after covering 120 slabs with nail varnish. Slabs were divided equally to the juice groups, 10 slabs per group. An iodide ion-specific electrode used to measure Ip on this window at baseline and after the immersion for a period of 20 min with 5 ml solution under constant agitation on an orbital shaker. The final Ip minus the baseline Ip gave the change in permeability, expressed as Ip 10^{-7} mol/L. Ca and F in the drinks were analyzed using a standard calcium ion-specific electrode or a fluoride ion-specific electrode respectively. Ca and F concentration recalculated and expressed in millimoles per litre and for F also in parts per million. The decrease in microhardness was greatest in Sprite Light (16.86±5.56 μm), followed by grapefruit juice (9.27±2.08 μm) and apple juice (8.98±1.92 μm), Perform (0.39±0.89 μm), drinking whey (-0.04±0.62 μm) and yoghurt (-0.61±0.38 μm). All differences were statistically significant from baselines

except for the Perform group (p=0.19) and drinking whey (p=0.86). All groups showed an increase in iodide permeability after 20 minutes immersion. Only grapefruit juice and lactate showed a significant difference (p=0.04). They found that the erosive capacity of different drinks, juices and foodstuffs were significantly associated with their acidity, pH values, as well as the baseline surface microhardness or iodide permeability values of the exposed enamel.

In previous studies, investigators studied titratable acidity using a long exposure time period to the erosive products. Theoretically, surface loss will increase with the increase titratable acidity as observed by investigators of previous studies. However, we will expect theoretically that for short exposure to erosive products that the buffering capacity will not have a detrimental effect on dental erosion because the erosive products will not be buffered completely in this short period. Therefore, it is important to check the effect of titratable acidity of erosive products with short repeatable exposures on dental tissues.

3.1.1.3 The effect of salivary pellicle on dental erosion

Saliva contains inorganic compounds and multiple proteins that affect conditions in the oral cavity and locally on the tooth surfaces, forming enamel pellicles (Slomiany *et al.*, 1986). Enamel pellicle is an acellular organic film directly covering the enamel surfaces of erupted teeth. It plays an important role in maintaining the integrity of the oral hard tissues (Slomiany *et al.*, 1986). The salivary pellicle layer, 10 μm thick, may protect the teeth against abrasion, erosion and demineralisation; it also affects the attachment of cariogenic microorganisms to enamel (Slomiany *et al.*, 1986). In addition, salivary buffer capacity is a factor of primary importance in maintaining oral homeostasis. The buffering system of the mouth depends on the total buffering capacity which consists of bicarbonate and phosphate systems and those based on proteins (Helm *et al.*, 1982). Buffer capacity of saliva during eating is related to the bicarbonate system as it utilizes carbon dioxide and water ($CO_2 + H_2O \leftrightarrow HCO_3^- + H^+$).

Meurman and Frank (1991a) exposed prismatic and aprismatic human enamel and unpolished or diamond-polished specimens of bovine enamel to a phosphoric acid containing cola beverage (pH 2.6) and citric or maleic acid containing sports drinks (pH 2.8 and 3.4, respectively) for 15-180 minutes. The prismatic human and bovine specimens showed a characteristic dissolution where initial erosion after 15 minutes

immersion was seen to affect specifically the prism sheath areas. Longer immersion caused dissolution of enamel prism cores followed by interprismatic areas. In bovine enamel maleic acid affected the surface ultra structure the least when compared with citric and phosphoric acids after 15-30 min of immersion, but thereafter no difference was observed between the acids in causing erosion. This study showed that the modification of the structure of enamel, including the removal of the outermost layer of enamel and/or salivary pellicle, had an impact on the progression of enamel dissolution/erosion. In addition, distribution and thickness of salivary pellicles may be responsible for the site specificity of dental erosion, and that pellicle does protect the teeth from erosion (Amaechi *et al.*, 1999b). In the *in vivo* part of the study, human enamel slabs were mounted on volunteers' teeth with orthodontic resin at eight sites of the mouth and left for 1 hour. Then, the slabs were stained with fluorescein isothiocyanate (FITC). The thickness of the pellicle was measured with confocal laser scanning microscopy (CLSM). The thickest pellicle (1.06 μm) occurred at the lower posterior lingual surface, while the thinnest pellicle (0.3 μm) occurred at the upper anterior palatal surface. In the lower arch, pellicle was significantly thicker in the anterior lingual surface compared with the anterior labial surface ($p \leq 0.01$) at a ratio of 2:1, while the posterior lingual surface had a significantly thicker pellicle than the posterior buccal surface ($p \leq 0.01$), also at a ratio of approximately 2:1. In the upper arch, the anterior palatal surface had a significantly thinner pellicle than the anterior labial surface ($p \leq 0.05$), while the posterior palatal surface had a significantly thinner pellicle than the posterior buccal surface ($p \leq 0.05$). Following the measurement of the pellicles thickness, slabs, either with pellicles "experimental" or without pellicles "control", were exposed to an erosive challenge with orange juice (pH 3.85) *in situ* 6 times daily for 5 minutes on each occasion for four days giving a total of two hours exposure to orange juice. Then, the slabs were examined with microradiography to check the mineral loss (Vol% mm). The level of erosion was significantly greater in the control slabs at all sites when compared with the experimental slabs. However, with pellicle, in the lower arch there were no statistically significant differences in the level of erosion between either the anterior labial and the posterior buccal surfaces or the anterior lingual and the posterior lingual surfaces. In the upper arch, the level of erosion was greater in the slabs placed in the anterior palatal sites than in the anterior labial sites ($p \leq 0.01$), with a similar observation when posterior palatal was compared with posterior buccal ($p \leq 0.01$), and anterior labial with posterior buccal ($p \leq 0.001$). The erosion level was not

significantly different between the anterior and the posterior palatal surfaces. When the erosion levels in both arches were compared together, the erosion level was significantly greater in the upper palatal sites compared with the lower lingual sites ($p \leq 0.001$), and was also greater in the lower posterior buccal site than in the upper posterior buccal site ($p \leq 0.05$). This study shows that the thickness of salivary pellicle varies within the dental arches and these variations determine the sites and severity of erosion within the arches. It also demonstrates that salivary pellicle protects against dental erosion. It is difficult to mimic the effect of salivary pellicles in *in vitro* studies; therefore an in situ design will be developed to study the effect of salivary pellicles on dental erosion.

As soon as the tooth erupts into the mouth a salivary pellicle begins to form on the surface. The pellicle is derived from specific salivary proteins and lipids that bind to the surface of the tooth (Mandel, 1989). The salivary proteins such as statherin, proline rich proteins, some histatins, and phospholipids comprise the initial pellicle (Mandel, 1989). The pellicle is continually regenerated throughout the life of the tooth in the mouth. The net effect with respect to erosion is that the pellicle forms a diffusion barrier, similar to a lipid/protein membrane, and protects the very outer surface against direct acid attack. Plaque bacteria then build up on the pellicle, forming a further diffusion barrier (Hara *et al.*, 2006).

3.1.1.4 The remineralisation effect of saliva on dental erosion

Saliva has a remineralisation role on teeth (Amaechi and Higham, 2001; Attin *et al.*, 2003). However, there was no evidence that human saliva, an artificial calcifying solution and fluoride mouth rinse applied after exposure to the erosive solution were effective in reducing the amount of tooth wear (Kelly and Smith, 1988). Amaechi and Higham (2001) used pre-eroded bovine incisors following a one hour immersion in orange juice. Enamel erosion was produced in each tooth by an hour immersion in the orange juice. The erosion was carried out at room temperature and at a low-speed (120 rpm) continuous magnetic stirring. Then, a control section and three experimental slabs were produced from each tooth. The three slabs were assigned randomly to one of three remineralising agents: clarified natural saliva, artificial saliva (methyl-p-hydroxybenzoate, 2.00 g/l; sodium carboxymethyl cellulose, 10.0 g/l; KCl, 8.38 mM; $MgCl_2.6H_2O$, 0.29 mM; $CaCl_2.2H_2O$, 1.13 mM; K_2HPO_4, 4.62 mM; KH_2PO_4, 2.40 mM;

fluoride, 0.022 ppm) and a remineralising solution (the same composition as the artificial saliva but without methyl-hydroxybenzoate and sodium carboxymethyl cellulose). All solutions had a pH of 7.2, a fluoride concentration of 0.022 ppm as this was the level in natural saliva, and were changed daily. Natural saliva was collected daily from the same individual at the same time of day. The specimens were incubated in their respective remineralising agents 20 ml/slab for 28 days in a cold room at 4°C to prevent microbial growth in natural saliva which does not contain antimicrobials. They used microradiography after a cycling period to measure the mean mineral loss and lesion depth for comparison. A significant ($p \leq 0.001$) amount of mineral was gained following exposure to each remineralising agent. Significantly less mineral loss and lesion depth were observed for the experimental groups compared with the control group ($p \leq 0.001$). This effect was greatest with the remineralising solution and least with artificial saliva. However, there were no significant differences between the three remineralising agents when they compared the mineral loss, but there was a significant difference in the lesion depth between remineralising solutions and this was least with the artificial saliva.

Kelly and Smith (1988) used six freshly extracted maxillary incisor teeth from the same patient which showed evidence of wear and with sufficient enamel present for testing. The teeth were cut along their long axes to produce a total of sixteen 1 mm thick slices. Each slice was tested at three sites along its length making a total of 48 test sites. Human saliva, an artificial calcifying solution ($CaCl_2$, $KC1_2$, $MgCl+6H_2O$, NaCl, $NaHCO_3$, NaH_2PO_4, H_2O, and glucose), a fluoride mouthrinse (0.05% NaF) and de-ionised water as a control were applied after exposure to the erosive solution and before brushing. The erosive agent used was a 50% dilution of a commercial lemon juice. The small difference between the control (de-ionised water) group and the three remineralising solution groups was not significant. However, the differences between the erosion only and abrasion only groups and between these groups and the control group were highly significant.

The remineralisation effect of saliva in reducing dental erosion has been controversial among researchers. This difference might be due to the different cycling techniques that were used or the different environment i.e. *in vivo* versus *in vitro*. Therefore, it is important to try to evaluate the remineralisation effect of saliva under conditions as close as possible to the *in vivo* situation.

3.1.1.5 The effect of abrasion on dental erosion

Amaechi *et al.*, (2003) investigated the influence of abrasion from oral soft tissues on softened enamel lesion remineralisation and erosion development in situ. Softened enamel lesions produced by ten minutes immersion in orange juice of ten human premolars were sectioned into four slabs each (two sections, one from the centre and one from one end of the lesion were used as controls and two were used as test slabs). The four slabs were assigned randomly to ten volunteers and attached to the palatal surfaces of their upper incisors. One of the two slabs from each tooth had the sound enamel surfaces painted with two coats of acid resistant nail varnish, while a protective device was constructed on the second slab to protect the softened enamel lesion from abrasion. The subjects used sugar-free chewing gum four times daily for 20 min on each occasion. They were asked to continue their normal dietary intake, and usual oral hygiene measures using fluoridated toothpaste twice daily. Neither the diet nor the type of fluoride toothpaste used were standardized or monitored. The slabs were removed after 28 days. Mineral loss and lesion depth in both control and test samples were quantified using transverse microradiography. Mineral loss was significantly lower in protected lesions ($p \leq 0.001$) but higher in unprotected lesions ($p \leq 0.001$) than in the control lesions. A similar pattern was observed with lesion depth. They concluded that:

"Dental erosion observed clinically is the combined effect of demineralisation of the tooth surface by an erosive agent and the subsequent abrasion of the demineralised surface by the action of the surrounding oral soft tissues and through food mastication. The abrasive effect of the oral soft tissues may contribute to the site-specificity of dental erosion".

In the study of Schweizer in 1978, it was found that it was possible to substantially abrade enamel, previously etched by orange juice with a toothbrush even without toothpaste, although the enamel loss was not as pronounced as produced with toothpaste. After softening with 5 min immersion in orange juice 2.4-4.9 μm could be removed *in vitro*. Davis and Winter (1980) found that *in vitro* acid attack (by a grapefruit/whole saliva mixture) caused both enamel and dentine losses and demineralisation of the remaining sub-surface tissue. They observed that if the remaining demineralised tissue was brushed even with only a toothbrush and water, accelerated abrasion occurred until the demineralised layers were removed. Although

the absolute quantities of tooth loss may be reduced *in vivo* due to the buffering capacity of saliva and the protective effect of salivary pellicle (Zahradnik *et al.*; 1976, Amaechi *et al.*, 1999b; Hannig and Balz, 1999), the rapid removal of tooth tissues, when an erosive attack is followed by toothbrush abrasion, was clearly demonstrated.

Eisenburger *et al.*, (2003) studied *in vitro* the wear of enamel after alternating or simultaneous cycles of erosion/abrasion. As an abrasive they used silica slurry instead of toothpaste. A statistically significant difference was observed between the surface losses produced by erosion and alternating erosion/abrasion. The amount of toothwear increased during the successive treatment cycles. However, in this *in vitro* study, a silica slurry instead of the regular fluoride toothpaste was used and, therefore, the toothwear produced could have been more pronounced. It was also found with in situ studies (Hooper *et al.*, 2003) that after toothbrushing with a fluoride toothpaste the enamel became more resistant to subsequent erosive/abrasive stimuli. On the contrary, in this study the opposite result was found, possibly due to lack of use of fluoride or due to the lack of salivary pellicle. These results could simulate eating after an erosive stimulus but not toothbrushing on softened enamel.

As toothbrushing after erosive food causes more enamel loss, it was interesting to investigate the period of remineralisation needed to re-establish the resistance of enamel against brushing abrasion after demineralisation with an acidic soft drink. Attin *et al.*, (2000) carried out an *in vitro* study where enamel specimens were immersed for 1 min in an erosive soft drink (Sprite Light) and after different periods of immersion into artificial saliva (0, 10, 60 and 240 min) they were brushed using an automatic brushing machine (100 strokes). It was found that even after a period of one hour of remineralisation, abrasion of previously eroded enamel was increased.

The same question was investigated by Jaeggi and Lussi, in 1999, who conducted an *in situ* study in order to quantitatively assess the influence of different exposure periods to the oral milieu on toothbrush abrasion of human enamel previously exposed to an acid attack. They found that toothbrush abrasion *in situ* was significantly lower ($p \leq 0.001$) after 60 min exposure to the oral environment than after zero min and therefore suggested that individuals at risk of erosive tooth wear should wait at least one hour before brushing their teeth after consuming erosive foodstuffs and beverages. A few years later Hooper *et al.*, (2003) carried out a single-blind, randomised, five-treatment cross-over study design in order to investigate erosion and abrasion on enamel and

dentine and to compare the abrasivity of two different toothpastes. In the abrasion/erosion regime the subjects drank orange juice four times per day and the specimens were brushed *ex vivo* immediately after the erosive incidence. During the study the volunteers followed their normal oral hygiene practices. Toothwear was more pronounced in the erosive/abrasive treatment than in the erosive or abrasive ones, although it did not reach statistical significance. Interestingly, there was no statistical significant difference between the surface losses produced by the two toothpastes of different abrasivity.

Similar findings were concluded by Turssi *et al.*, (2004), who investigated the *in situ* abrasion of eroded dental hard tissues by a whitening dentifrice compared to a normal paste found that independently of the toothpaste used the toothbrush abrasion of acid-treated specimens was always more than those of untreated specimens. However, because the erosive episodes were performed extra-orally, they were not counterbalanced by oral clearance, as a result of the stimulation of increased salivary flow, and by the buffering capacity of saliva (Millward *et al.*, 1997).

Finally, except for the aforementioned *in vitro* and *in situ* studies, Al- Dlaigan *et al.* (2002) looked at the influence of oral hygiene practices on the prevalence of dental erosion in a group of 418, 14-year-old schoolchildren in Birmingham, UK and it was found that there was a higher incidence of dental erosion in children who brushed their teeth always after meals. The authors claimed that it was possible, as far as toothwear was concerned, that the abrasive content of the toothpaste could be more important than the fluoride level. However, as the investigators point out, in this study there were numerous variables and multiple tests of significance. Therefore, there is the possibility of spurious results that may have occurred by chance.

Dental erosion increases when it is associated with abrasion caused by soft oral tissues or external sources i.e. toothbrush as described above. Therefore, investigation of the effect of manual and electrical brushing and comparing their effects versus not brushing was important to improve our knowledge in developing a cycling technique to create erosive lesions in *in vitro* situations. This will improve our understanding to the *in situ* scenario results.

3.1.1.6 The effect of fluoride on dental erosion

Attin *et al.* (2003) investigated the effect of mineral supplements added to citric acid (1% pH 2.21) on enamel erosion under controlled conditions in an artificial mouth on enamel of bovine teeth. The study included 13 experimental groups (n=12). In group one citric acid only was used (control). In groups 2—10 of calcium, phosphate or fluoride at various low concentrations were mixed with the citric acid. In groups 11—13 the citric acid was supplemented with a mixture of calcium, phosphate and fluoride. The specimens were rinsed with the respective solution for one minute, followed by a remineralisation period with artificial saliva for one minute. The specimens were cycled through this alternating procedure five times within 10 min. After cycling through this de- and remineralisation procedure, the specimens were rinsed for eight hours with artificial saliva. The de- and remineralisation cycle (10 min) was repeated three times for each specimen interrupted by eight hours remineralisation periods. Surface microhardness was used for analysis. A significant difference for all microhardness values of the experimental groups ($p \leq 0.05$) was observed compared with the baselines. Addition of calcium to the citric acid solution resulted in significantly lower microhardness compared with the controls. As well as the addition of phosphate, the addition of fluoride caused significantly lower hardness reduction than for the controls. No significant differences were observed among the groups treated with citric acid and different phosphate concentrations. The same effect was seen when the citric acid was supplemented with fluoride. The combination of calcium, phosphate and fluoride added to the citric acid also led to significantly lower hardness loss compared to controls. The least hardness loss was recorded for the combination of calcium, phosphate and fluoride added to the citric acid. In a similar way, Lussi *et al.* (1993) found that the increased erosive capacity of different drinks, juices and foodstuffs were associated with lower phosphate and fluoride contents.

Amaechi *et al.* (1998a) studied the effect of xylitol, fluoride and xylitol/fluoride combined on the erosion of bovine dental enamel by pure orange juice *in vitro*. Ten bovine incisors were used. Four erosive agents were prepared. Pure orange juice only; pure orange juice plus either xylitol (25%) fluoride (0.5 ppm); or xylitol/fluoride (25% and 0.5 ppm respectively) were used. Mineral loss was quantified using a two-step image analysis system. The only significant difference ($p \leq 0.05$) in mineral loss was found when the xylitol/fluoride group was compared with pure orange juice.

Fluoride has an impact effect on dental erosion as an ion present in saliva (Amaechi and Higham, 2001) or as compound i.e. toothpastes and mouthrinse (Kelly and Smith, 1988; Attin et al., 2003). Therefore, developing preventive measures against dental erosion using fluoride seemed a wide area in need of further investigation and development.

3.2 Study-4: The effect of toothpastes containing different concentrations of sodium fluoride on erosion of human enamel under pH cycling conditions *in vitro*

3.2.1 Aim

The aim of our study was to investigate the effect of fluoride in preventing mineral loss of enamel under acidic conditions which would mimic a moderate erosive challenge, using a pH cycling model. The null hypothesis, there is no difference in tooth surface loss of enamel with different concentrations of fluoride in toothpaste.

3.2.2 Materials and Methods

Twenty-one enamel slabs from intact human premolars were divided into three groups. Each group received daily erosive cycling procedures for a period of 16 days. In addition, each group was treated with one of the test toothpastes (0 ppm F, 1100 ppm F, or 1385 ppm F). Toothpastes were blinded to the study personnel and codes only released by the sponsor at the end of the study. Enamel slabs were allocated to groups using a coded randomisation list provided by the sponsor. The following section details the preparation of the slabs, experimental treatment and test methods that were used in this study.

3.2.2.1 Slab Preparation

This section was discussed in section 2.4.1 (page 25).

3.2.2.2 Storage of Enamel Slabs

Once the slabs had been prepared, they were kept moist in micro-centrifuge tubes containing de-ionised distilled water and left at room temperature to prevent dehydration of the slabs. This process was repeated after finishing the experiment.

3.2.2.3 Surface Profilometry (SP)

In this study, a new software version was used which was validated by the manufacturer and compared to the previous version. Baseline measurements of the the surface profiles

of the slabs were obtained using SP (ProScan 2000, version 2.1.1.8, Scantron Industrial Products Limited, Somerset, England) to ensure the surface flatness. The measurement was achieved by placing the sample on a key stage on the Scantron ProScan. The sensor used had a measuring range of 300 μm with an average distance of 5 mm from the surface. The measuring range was set to 150 μm. The resolution of the sensor was 10 nm and the spot size was 8 μm. The measure rate/frequency was set to 300 Hz to give a minimum intensity of 5% of reflected light for analysis. A step size of 0.01 mm was used during scanning. After scanning, the flatness of the surface using cross-sectional views was checked. Slabs that were not flat were ground once again as described previously and were then checked again with the ProScan. Slabs with exposed dentine after this process were excluded. The surface of the enamel slabs were then covered with nail varnish except for a small window of 1x2 mm size in the middle of each slab. At days 4, 8, 12 and 16 the nail varnish was removed using acetone and the same procedure of scanning was repeated to check the average depth of surface loss (SL) of the exposed area. Using the Proscan software package, three areas of interest were defined (Figure 2.2) and the average depths of the eroded areas were calculated. Slabs were covered with nail varnish carefully after each scan during the re-cycling period.

3.2.2.4 Experimental Protocol/Regime

The study consisted of three treatment groups each containing seven enamel slabs. A special tray with eight holes was used to hold the slabs of each group. Slabs were immersed under static conditions for two minutes, five times daily, in fresh 200 ml aliquots of 0.3% citric acid (adjusted to pH 3.6) for 16 days giving a total exposure time of 160 minutes. In addition, the slabs were dipped twice daily in toothpaste slurries for two minutes, once before cycling with citric acid (morning) and then after cycling (evening). The concentrations of fluoride used were 0, 1100 ppm F and 1385 ppm F. Fresh slurries were prepared daily by adding three grams of toothpaste to 10 ml of de-ionised distilled water and mixed using a stirrer and were used fresh. Slabs were incubated in artificial saliva in between dippings and over night at 37°C. Table 3.1 shows the composition of the artificial saliva. Artificial saliva was changed daily to prevent any contamination or bacterial growth. A sixty minute gap was left between daytime erosive challenges and between dipping in toothpastes and erosive challenges.

Before and after dipping in the erosive solutions the slabs were rinsed with de-ionised water.

3.2.2.5 Statistical Analysis

SPSS for Windows software (V. 13, SPSS Inc., 1989-2002; LEAD technologies Inc., 1991-2000) was used for data analysis. Significant level was "*p*" values at 5% level using Student paired t-test. Confidence intervals and "*p*" values were used to interpret the results. Sample size of 7-8 was considered acceptable for the *in vitro* studies in this chapter considering our preliminary studies in chapter 2.

Table 3.1: Artificial Saliva contents used in our cycling technique (Almståhl & Wikkström 2003). pH = 6.80±0.05

Salt	Concentration g/L
Sodium Chloride	0.233
Sodium Carbonate	2.352
Calcium Chloride	0.014
Potassium Chloride	2.544
Potassium di-hydrogen phosphate	0.3864

3.2.3 Results

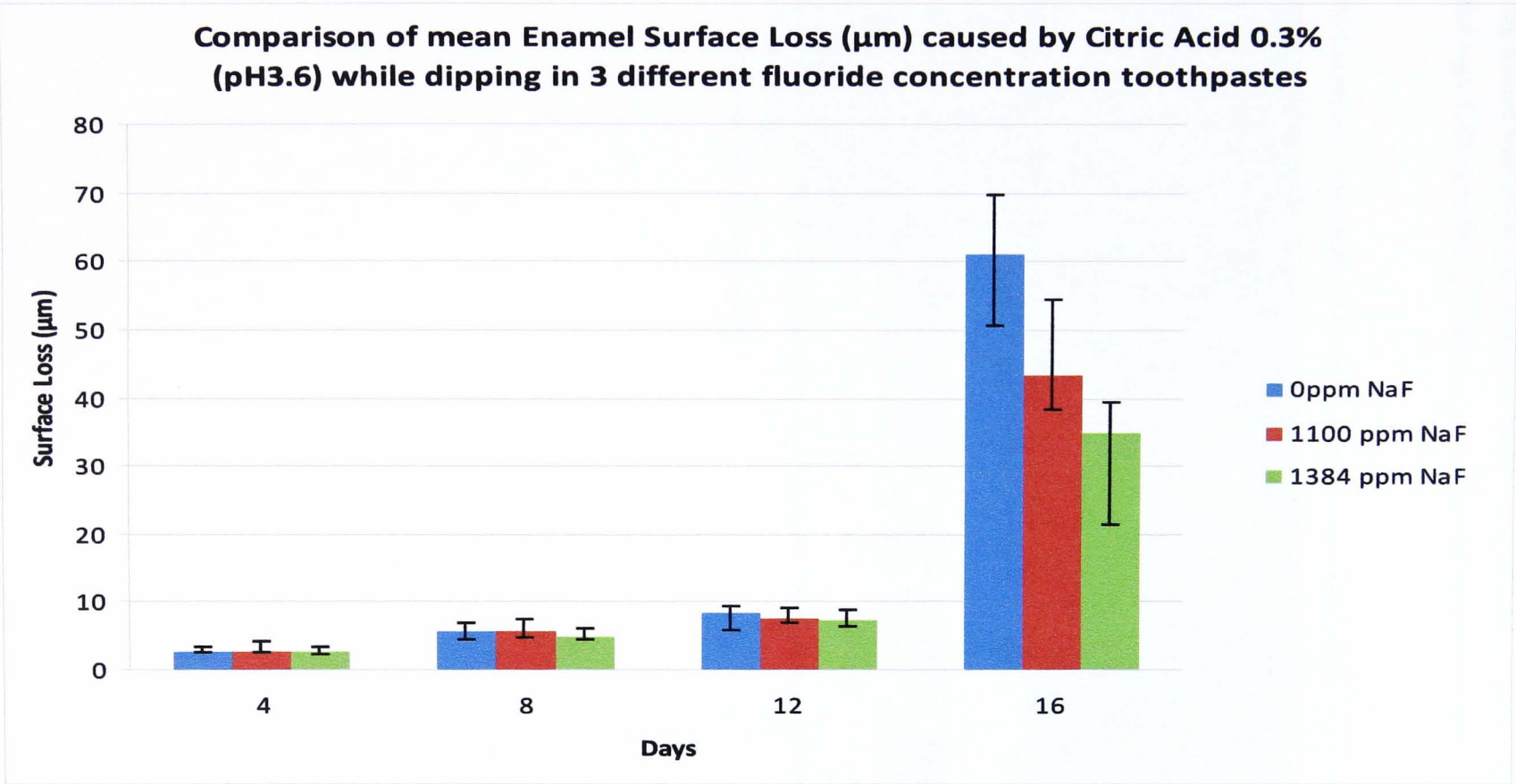
All treatment groups showed surface loss progression at all time points during the experiment (Table 3.2 and Figure 3.1). The extent of surface loss from baseline to day 12 was modest. However a significant increase was observed across all groups between days 12 and 16. Between-treatment comparisons at day 16 indicated a clear fluoride dose response effect with the least mineral loss occurring for the highest (1385 ppm F) toothpaste, followed by the 1100 ppm F group.

Table 3.2: Mean Change of Surface Loss from baseline caused by 0.3% Citric Acid (pH 3.6) while dipping in three different fluoride concentration toothpastes (n=7 slabs per group).

Group Statistics	Day of Scanning	Mean Change from Baseline	SD	SE
0 ppm F	Day 4	2.81	0.37	0.14
1100 ppm F	Day 4	2.83	1.18	0.45
1385 ppm F	Day 4	2.71	0.57	0.22
0 ppm F	Day 8	5.68	1.07	0.40
1100 ppm F	Day 8	5.76	1.53	0.58
1385 ppm F	Day 8	5.00	1.04	0.39
0 ppm F	Day 12	8.33	0.97	0.37
1100ppm F	Day 12	7.38	1.37	0.52
1385ppm F	Day 12	7.67	1.31	0.49
0 ppm F	Day 16	61.19	8.50	3.21
1100 ppm F	Day 16	43.44	10.94	4.14
1385 ppm F	Day 16	34.98	4.29	1.62

Source: Appendix 24

Figure 3.1: Comparison of Enamel Surface Loss caused by 0.3% Citric Acid (pH 3.6) with the three different fluoride concentration toothpastes. Error bars are standard deviation bars.



The group treated with the 0 ppm toothpaste showed a significantly higher mineral loss compared with 1385 ppm NaF ($p \leq 0.05$) and 1100ppm NaF ($p \leq 0.05$) (Table 3.3) at day 16.

Table 3.3: Comparison of the difference of mean surface loss (µm) with the three toothpastes at day 16.

Groups	Mean Diff	SD of Mean Diff	SE of Mean Diff	(2-tailed Sig.)	95% Confidence Interval of the Difference	
					Lower	Upper
0 ppm F vs 1385 ppm F	26.21	3.60	3.60	0.01*	18.360	34.052*
0 ppm F vs 1100 ppm F	17.74	5.24	5.24	0.03*	6.328	29.158*
1385 ppm F vs 1100 ppm F	-8.46	4.44	4.44	0.07	-18.145	1.219

***Significantly different**

-ve values favours the first named treatment

3.2.4 Discussion

In this section, the discussion of the model used and of the results is presented.

3.2.4.1 In vitro Model

In vitro models of various designs have been used for the demineralisation or the production of artificial dentine caries lesions. *In vitro* studies are highly controlled (ten Cate, 1994) but suffer from limitations as the dentine substrates are non-vital thus, cannot reproduce biological responses to an acid challenge. In addition, the conditions in the oral environment cannot be simulated *in vitro* accurately.

With regard to demineralisation systems, the majority have been adapted from enamel models and can be divided to partially saturated buffers (Hopppenbrouwers *et al.*, 1987; ten Cate *et al.*, 1998) acidified gels (Arends *et al.*, 1990; Ruben and Arends, 1993; Wefel *et al.*, 1995) and pH cycling models (Almqvist *et al.*, 1990, Dunipace *et al.*, 1994).

In our study we used a pH cycling model that has been used successfully for the investigation of enamel demineralisation but still needs further evaluation. *In vitro* models of various designs have been used for the evaluation of tooth surface loss due to the interaction of erosion and abrasion. For simulating intra-oral erosion it is desirable to assess the effects on native tooth surfaces, however, for precise assessment of the tooth surface loss or for creating a reference surface for the *in vitro* studies the outer surface needs to be removed (Ganss *et al.*, 2000).

Studies *in vitro* offer a well controlled environment (Hall *et al.*, 1998) but they lack any influence from the numerous variables encountered in the oral environment, most of which would provide a protective effect against acid erosion such us rapid clearance of the acid substrate by saliva, saliva flow rate and composition, formation of pellicle, tooth anatomy and structure, etc (West *et al.*, 1998; Hannig, 1999; Amaechi *et al.*, 1999b; Wetton *et al.*, 2006). Our model design did not take into consideration the effect of these factors on dentine surface loss.

The design of this study attempted to simulate *in vitro* the most commonly practised oral hygiene habits. We used an erosion model that has been used successfully in our preliminary work for the investigation of enamel and dentine surface loss but still needs further evaluation for dentine *in vitro* studies.

3.2.4.2 Discussion of Results

This was an exploratory study using a longitudinal cycling *in vitro* erosion model to investigate the effect of three different concentrations of fluoridated toothpastes on tooth surface loss under erosive conditions.

In developing the experimental design we attempted to provide a moderate erosive challenge to promote sufficient levels of surface loss to satisfactorily explore the effects of preventive treatments i.e. fluoridated toothpaste on enamel surface loss progression. The aim was to establish a method that could be used to evaluate the relative performance of toothpaste formulations ahead of more complex *in situ* or randomised clinical trial investigations.

Previous researchers investigating erosion *in vitro* have employed a six times daily erosive challenge lasting for five minutes on each occasion (Amaechi *et al.*, 1998a & 1999c; Amaechi & Higham, 2001; Kelly and Smith, 1988). We believe this is probably an over-estimation of the "real life" situation and represents a worst case scenario indicative of individuals who may have an underlying medical condition (e.g GORD) or irregular dietary habits. In a previously published *in vitro* study, it was found that mimicking the wine tasters sample of five to 50 wines each day, simply holding the wine in their mouth from 15 to 60 seconds was sufficient time for widespread erosion to occur if no other factors intervened (Mok *et al.*, 2001). Therefore we have elected to use a five times daily dipping regime (soaking without agitation) with each dipping occasion lasting for two minutes only, giving a total acid challenge of 10 minutes per day, with appropriate remineralisation periods in between.

By cycling over a period of 16 days, this model has allowed the study of longitudinal effects which again are believed to be more representative of the clinical situation. The results have clearly showed that surface loss progression was relatively slow over the first 12 days of the experiment but that a large and significant increase occurred between days 12 and 16. It was during this phase that the effect of fluoride level/concentration used became most pronounced. It is hypothesized that a critical threshold level might exist beyond which the demineralisation phase of enamel is accelerated. The erosive challenge gradually increases the porosity of the enamel matrix and that over time this increase in porosity allows even greater depth of acid penetration overwhelming any remineralisation effects from the toothpaste or artificial saliva. At

this point there is an increase in extent of demineralisation generating a larger softened surface zone which is easily removed from the underlying sound tissue. The results are consistent with existing data showing that extensive exposures to fluoride treatment using toothpaste reduce the erosive mineral loss values for enamel and dentine (Ganss *et al.*, 2001). More research on this hypothesis is required, but it would seem prudent that such studies should be carried out over a longer period of time, for example for >21 days in order to fully understand the erosive process and the beneficial effect of fluoridated toothpastes, in particular their dose response and the impact of fluoride source.

In an *in situ* study Zero *et al.*, (2006) found a beneficial effect of an experimental toothpaste containing 1,100 ppm F and 5% KNO_3 compared to a toothpaste available on the market with 1,100 ppm F. In addition, Bartlett *et al.* (1994) and Magalhaes *et al.* (2007) showed a beneficial effect of fluoride dentifrice. These researchers used a more severe and longer attack (e.g. 25 min with grapefruit juice with its low pH and high buffering capacity, or 10min and additional 30 min exposure to acidic drinks).

Lussi *et al.* (2008) evaluated the hardness of enamel and reported a significant difference of Pronamel toothpaste when compared to the negative control, but the dipping time (three min at pH 4.0), laboratory procedure and method of assessment of erosion were different than in our study.

In our study there were two applications of the toothpaste slurry before and after the erosion challenge. In previous studies incubation of enamel or dentine specimens in toothpaste slurry prior to softening seems to be more favourable than post exposure incubation (Hughes *et al.*, 2004; Ponduri *et al.*, 2005; Lussi *et al.*, 2008). This may be due to some incorporation of material into and/or deposition of material onto the enamel surface, most probably as a CaF_2 -like material, which will lead to less softening than in the absence of this layer (Ganss *et al.*, 2001; Lussi *et al.*, 2008). The design of this study attempted to simulate *in vitro* the most commonly practised oral hygiene habits.

In the initial stage of erosion the lesion could be remineralised but in later stages when the surface is completely lost, the erosion process cannot be reversed (Amaechi and Higham, 2005). The amorphous calcium phosphate is able to release calcium and phosphate ions to maintain the supersaturated state, thus enhancing the remineralisation process (Reynolds, 1997). This is probably the better outcome in the present study for

the group with the fluoridated toothpaste plus tooth mousse even from the first week of the study and the less DSL that was exhibited during the whole pH cycling period was the result of the additive effect of F and CPP-ACP.

There was a clear fluoride dose response evident in this study with the least surface loss observed when the 1385 ppm F toothpaste was used. (The difference compared with the 1100 ppm F paste was directional (p=0.07) but not statistically significant). The role of fluoridated toothpastes in the prevention of caries is well established but researchers have not yet attempted to show a similar protective effect of fluoride on erosion in a clinical situation. Other than a lack of validated clinical methods and associated assessment tools such studies present clear 'ethical difficulties'. Therefore use of *in vitro* and *in situ* models represents the current 'state of the art' for elucidating mechanistic understanding, and fluoride (in toothpaste) clearly appears to have a protective effect. The mechanism by which this might happen is hypothesized as either inhibition of demineralisation of the enamel surface or remineralisation of the softened surface of enamel before its loss.

Also, tooth surface loss as manifested clinically is a combination of both erosion and the abrasive challenge due mainly to toothbrushing. It would be interesting to study the fluoride dose response in a model that would incorporate brushing of the enamel surfaces after exposure to an erosive challenge. In addition, extending the cycling period might explain the threshold of increase tooth surface loss. Further investigation of the specific cycling model for enamel and dentine surface loss should be carried out.

3.2.5 Conclusion

The null hypothesis was rejected with the results of this study. Even though these are preliminary results, carefully extrapolated, it would seem sensible to recommend toothpastes with a higher level of fluoride for those individuals who are particularly predisposed to tooth surface loss or have risk factors / habits that suggest the need for additional protection from erosive challenges.

3.3 Study-5: A Longitudinal *in vitro* Study Investigating the Effect of Sodium Fluoride and Toothbrush Abrasion on Surface Loss of the Dental Hard Tissues under Erosive Challenge

3.3.1 Aims

1. To investigate the effect of fluoride on tooth surface loss of dental hard tissues using a 21 day pH cycling regimen. The null hypothesis, there is no difference between different concentrations of fluoride on tooth surface loss of dental hard tissues.
2. To investigate the effect of brushing on tooth surface loss of dentine using a 21 day pH cycling regimen. The null hypothesis, there is no difference between different concentrations of fluoride on the abrasion of dental hard tissues.
3. To investigate the effect of two different artificial salivas on tooth surface loss of human enamel. The null hypothesis, there is no difference between two different artificial salivas in pH cycling model on tooth surface loss of dental hard tissues.
4. To investigate whether using enamel slabs in a narrow baseline range of KMH will improve the results of the study by reducing the effects of outliers. The null hypothesis, there is no difference when using standardised microhardness as inclusion criteria for dental hard tissue sample on the outcome of tooth surface loss of these samples after exposure to pH cycling regime.

3.3.2 Material and methods,

This study builds on a previous investigation which examined the effect of fluoride and brushing on tooth surface loss on human enamel for a period of 16 days. In this study, the cycling period was extended to 21 days and the experiment involved testing both enamel and dentine. In addition, Knoop Microhardness (KMH) was used as one of the inclusion criteria in an attempt to eliminate the effect of the baseline differences of the slabs used.

Forty-eight enamel slabs were distributed randomly into six groups using a randomisation table. The same number and procedure were performed for dentine slabs. This section explains the materials and methods used in this study.

3.3.2.1 Slab Preparation

This section was discussed in section 2.4.1 (page 25).

3.3.2.2 Storage of Enamel Slabs

Once the slabs had been prepared, they were kept moist in micro-centrifuge tubes containing de-ionised distilled water and left at room temperature. This process was repeated after finishing the experiment.

3.3.2.3 Test Methods

3.3.2.4 Knoop Microhardness (KMH)

Baseline measurements were recorded using Knoop microhardness as an inclusion criterion. Microhardness was assessed using a computer-aided Duramin Indenter Machine (Struers A/S, DK 26-10, Denmark). The indentations were made using a Knoop diamond under a 100 g load for 30 sec for enamel and a 50 g load for 30 sec for dentine. The length of indenter penetration was measured by means of an image analysis system. Five indentations, spaced 50 μm apart, were made for each slab and the mean was estimated. The area to have erosive treatment was avoided by covering it with tape. The length of each indent was recorded three times and the mean was determined. An average of 62-68 μm and 80-95 μm of indent length was considered as an acceptable inclusion criterion for enamel and dentine respectively.

3.3.2.5 Surface Profilometer (SP)

This section was discussed in section 3.2.2.3 (page 67).

3.3.3 Blindness and Randomisation

3.3.3.1 Blindness

The three toothpastes used in the study were coded and the code was kept by the study sponsor. At the end of the study, the results were sent to the study sponsor and then the code was released to the study investigator.

3.3.3.2 Randomisation

Enamel slabs were randomly allocated to each study group using the toss of a coin.

3.3.3.3 Experimental Protocol/Regime

Each group in the study consisted of eight slabs of enamel/dentine. There were seven groups of enamel and six groups of dentine in the study.

Table 3.4 shows the distribution of the groups. A special tray with eight holes was used to hold the slabs which were embedded in resin blocks. The slabs were immersed under static conditions for two minutes five times daily in 0.3% citric acid (pH 3.6) for 21 days making a total exposure time of 210 minutes. In addition, the slabs were dipped twice daily in one of the test toothpastes for two minutes with or without brushing, once before cycling with citric acid and then after cycling. The concentrations of fluoride used were 0ppm NaF, 1450ppm NaF (Sensodyne Pronamel®), and 1500ppm NaF (Colgate Sensitive®). Toothpastes were prepared by adding three grams of toothpaste to 10 mL of de-ionised distilled water and mixed using a stirrer.

Toothbrushes of medium coarse bristles (Sainsbury's, Sainsbury's Supermarket Ltd, London, Produced in China) were used. Fifteen strokes of 200 g weight were applied using a brushing machine (NEL-BSI Dentifrice Test Machine, UK) for the groups received brushing during dipping in the toothpastes.

Table 3.4: Distribution of groups in the study

Experimental Groups		Dipping in toothpaste with brushing		Dipping in toothpaste without brushing
		Artificial Saliva-1ʃ	Artificial Saliva-2*	Artificial Saliva-1
Enamel	1- 0 ppm F (Placebo)	Group-1	Group-4	Group-5
	2- 1450 ppm F (Sensodyne Pronamel) (Test)	Group-2		Group-6
	3- 1500 ppm F (Colgate Sensitive) (Control)	Group-3		Group-7
Dentine	1- 0 ppm F (Placebo)	Group-1		Group-4
	2- 1450 ppm F (Sensodyne Pronamel) (Test)	Group-2		Group-5
	3- 1500 ppm F (Colgate Sensitive) (Control)	Group-3		Group-6

∫ Table 3.5: Artificial Saliva-1:

Salt	Concentration g/L
Sodium Chloride	0.233
Sodium Carbonate	2.352
Calcium Chloride	0.014
Potassium Chloride	2.544
Potassium di-hydrogen phosphate	0.3864

***Table 3.6: Artificial Saliva-2:**
It consists of 2 solutions:

1) remineralisation solution during day time:

Salt	Concentration 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

Preparation was the addition of 1.8 ml 1 mol/L HCl to 900 ml distilled water. Then, the above components were added and stir red until all have dissolved. The pH was adjusted to 6.8 by adding KOH solution. Then the solution was topped with distilled water to make up to 1 L. The solution was kept in a refrigerator and used it within a few days otherwise a fresh solution was made up. This solution was advised by Dr P Shellis specifically for this study in order to eliminate any precipitation on the enamel surface.

2) Storage solution during night-time:

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

Preparation was the addition of 1.4 ml 1 mol/L HCl to 900 ml distilled water. Then the above components were added and stir red until all have dissolved. pH was adjusted to 6.8 by adding KOH solution. Then the solution was topped with distilled water to make up to 1 L. The solution was kept in a refrigerator and used it within a few days otherwise a fresh solution was made up. This solution was advised by Dr P Shellis specifically for this study in order to avoid any precipitation on the enamel surface.

Slabs were incubated in artificial saliva in between dippings and over night in artificial saliva at 37°C. Artificial saliva was changed daily to prevent any contamination or bacterial growth. A 60 minute gap was left between daytime erosive challenges and between dippings in toothpastes and the erosive challenges. Before and after dipping in the erosive solutions the slabs were rinsed with de-ionised water.

During the cycling period, the slabs were analysed with the scanning profilometer to measure the amount of surface loss at days 8, 12, 14, 16 and 21.

3.3.3.4 Statistical Analysis

An SPSS statistical software package for Windows, version 13.0 (SPSS Inc., 1989-2002; LEAD technologies Inc., 1991-2000) was used for data analysis. Student paired t-test was used to compare between groups. Significance was considered when $p \leq 0.05$. Confidence interval and *"P"* values were used to interpret the results.

3.3.4 Results

3.3.4.1 Enamel

3.3.4.1.1 Effect of different fluoridated toothpastes on enamel erosion:

Sensodyne Pronamel toothpaste showed less tooth surface loss than both Colgate Sensitive and placebo toothpastes at all timepoints in the groups that were dipped with or without brushing in toothpastes as shown in Figure 3.2. This difference was significant ($p \leq 0.05$) on day 21 (Table 3.7, Figure 3.3, Table 3.8, Appendix 27).

Figure 3.2: Comparison of the effect of three different concentrations of fluoride toothpastes on human enamel using dipping only twice daily in the toothpaste and using a pH cycling technique (0.3% citric acid pH=3.6) for a period of 21 days(±SD).

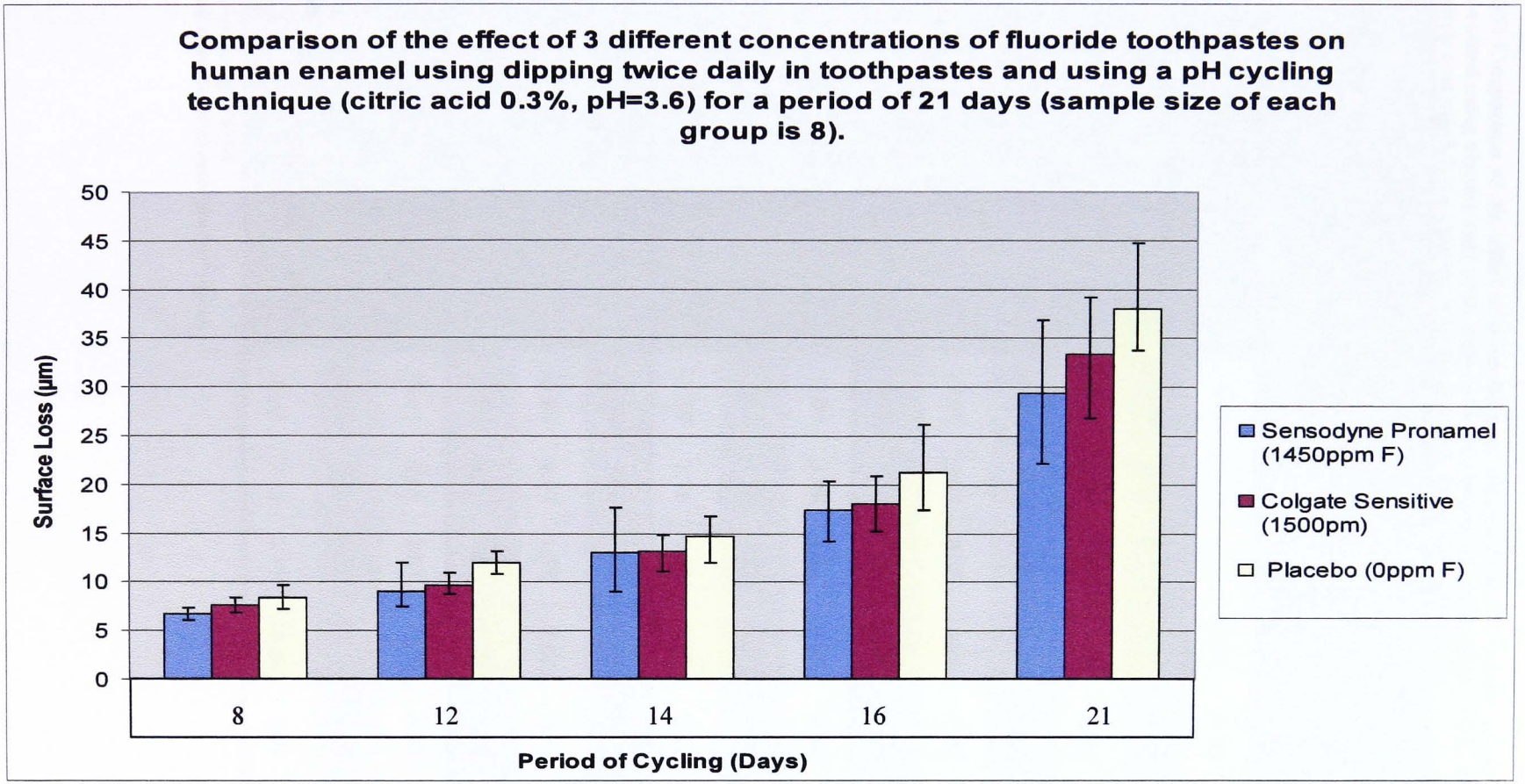


Table 3.7: Comparison of the effect of three different concentrations of fluoride toothpastes on human enamel using dipping only twice daily in the toothpaste and using a pH cycling technique (0.3% citric acid pH=3.6) for a period of 21 days (sample size of each group is 8).

Dipping Groups		Diff of Mean (µm)	SD	SE	95% Confidence Interval of the Difference		Sig. (2-tailed) **
					Lower	Upper	
Day-8	Sensodyne Pronamel (1450 ppm F) VS Colgate Sensitive (1500 ppm F)	-0.83	0.66	0.23	-1.37	-0.28	0.01*
	Sensodyne Pronamel (1450 ppm F) VS Placebo	-1.62	1.10	0.39	-2.54	-0.70	0.00*
	Colgate Sensitive (1500 ppm F) VS Placebo	-0.79	0.92	0.33	-1.56	-0.02	0.05*
Day-12	Sensodyne Pronamel (1450 ppm F) VS Colgate Sensitive (1500 ppm F)	-0.69	2.00	0.71	-2.36	0.99	0.36
	Sensodyne Pronamel (1450 ppm F) VS Placebo	-2.91	1.85	0.65	-4.46	-1.37	0.00*
	Colgate Sensitive (1500 ppm F) VS Placebo	-2.23	1.36	0.48	-3.36	-1.09	0.00*
Day-14	Sensodyne Pronamel (1450 ppm F) VS Colgate Sensitive (1500 ppm F)	-0.07	3.48	1.23	-2.98	2.83	0.96
	Sensodyne Pronamel (1450 ppm F) VS Placebo	-1.67	3.18	1.13	-4.33	0.99	0.03*
	Colgate Sensitive (1500 ppm F) VS Placebo	-1.59	1.76	0.62	-3.07	-0.12	0.04*
Day-16	Sensodyne Pronamel (1450 ppm F) VS Colgate Sensitive (1500 ppm F)	-0.66	3.58	1.26	-3.65	2.33	0.62
	Sensodyne Pronamel (1450 ppm F) VS Placebo	-3.86	4.47	1.58	-7.60	-0.12	0.05*
	Colgate Sensitive (1500 ppm F) VS Placebo	-3.20	4.28	1.51	-6.79	0.38	0.07
Day-21	Sensodyne Pronamel (1450 ppm F) VS Colgate Sensitive (1500 ppm F)	-9.63	5.86	2.07	-14.53	-4.72	0.00*
	Sensodyne Pronamel (1450 ppm F) VS Placebo	-8.64	5.86	2.07	-13.53	-3.74	0.00*
	Colgate Sensitive (1500 ppm F) VS Placebo	-4.63	7.15	2.53	-10.60	1.34	0.11

*** Statistically significant**

**** paired t-test used to measure "*p*" values**

Figure 3.3: Comparison of the effect of three different concentrations of fluoride toothpastes on human enamel using brushing twice daily in the toothpastes and using a pH cycling technique (0.3% citric acid, pH=3.6) for a period of 21 days (±SD).

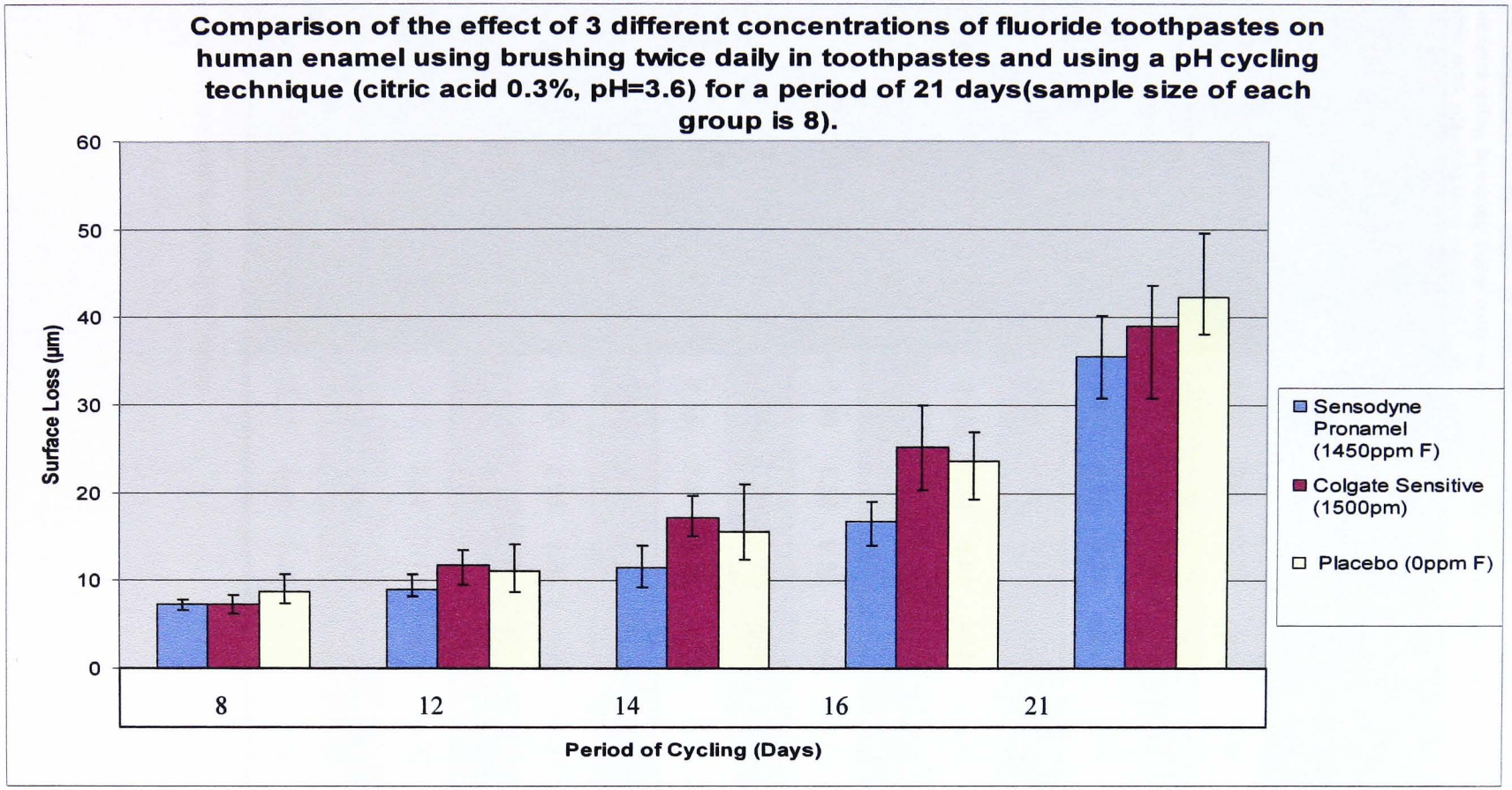


Table 3.8Comparison of the effect of three different concentrations of fluoride toothpastes on human enamel using brushing twice daily in the toothpastes and using a pH cycling technique (0.3% citric acid, pH=3.6) for a period of 21 days (±SD).

Dipping Groups		Diff of Mean (µm)	SD	SE	95% Confidence Interval of the Difference		Sig. (2-tailed) **
					Lower	Upper	
Day-8	Sensodyne Pronamel (1450 ppm F) VS Colgate Sensitive (1500 ppm F)	0.01	0.65	0.23	-0.54	0.55	0.98
	Sensodyne Pronamel (1450 ppm F) VS Placebo	-1.43	1.06	0.37	-2.32	-0.55	0.01*
	Colgate Sensitive (1500 ppm F) VS Placebo	-1.44	0.80	0.28	-2.11	-0.77	0.00*
Day-12	Sensodyne Pronamel (1450 ppm F) VS Colgate Sensitive (1500 ppm F)	-2.88	1.34	0.47	-4.00	-1.76	0.00*
	Sensodyne Pronamel (1450 ppm F) VS Placebo	-2.23	2.28	0.81	-4.13	-0.32	0.03*
	Colgate Sensitive (1500 ppm F) VS Placebo	0.65	2.18	0.77	-1.17	2.47	0.43
Day-14	Sensodyne Pronamel (1450 ppm F) VS Colgate Sensitive (1500 ppm F)	-5.43	1.50	0.53	-6.68	-4.17	0.00*
	Sensodyne Pronamel (1450 ppm F) VS Placebo	-4.06	2.24	0.79	-5.93	-2.20	0.00*
	Colgate Sensitive (1500 ppm F) VS Placebo	1.36	2.81	0.99	-0.99	3.71	0.21
Day-16	Sensodyne Pronamel (1450 ppm F) VS Colgate Sensitive (1500 ppm F)	-8.39	3.30	1.17	-11.15	-5.63	0.00*
	Sensodyne Pronamel (1450 ppm F) VS Placebo	-6.85	2.96	1.05	-9.33	-4.37	0.00*
	Colgate Sensitive (1500 ppm F) VS Placebo	1.54	3.72	1.32	-1.57	4.65	0.28
Day-21	Sensodyne Pronamel (1450 ppm F) VS Colgate Sensitive (1500 ppm F)	-3.52	3.36	1.19	-6.33	-0.72	0.02*
	Sensodyne Pronamel (1450 ppm F) VS Placebo	-6.71	4.93	1.74	-10.83	-2.58	0.01*
	Colgate Sensitive (1500 ppm F) VS Placebo	-3.19	4.07	1.44	-6.59	0.21	0.06

***Statistically significant**

**** Independent t-test used to measure "*p*" values**

3.3.4.1.2 Comparison of the effect of brushing versus dipping using toothpastes on TSL of enamel:

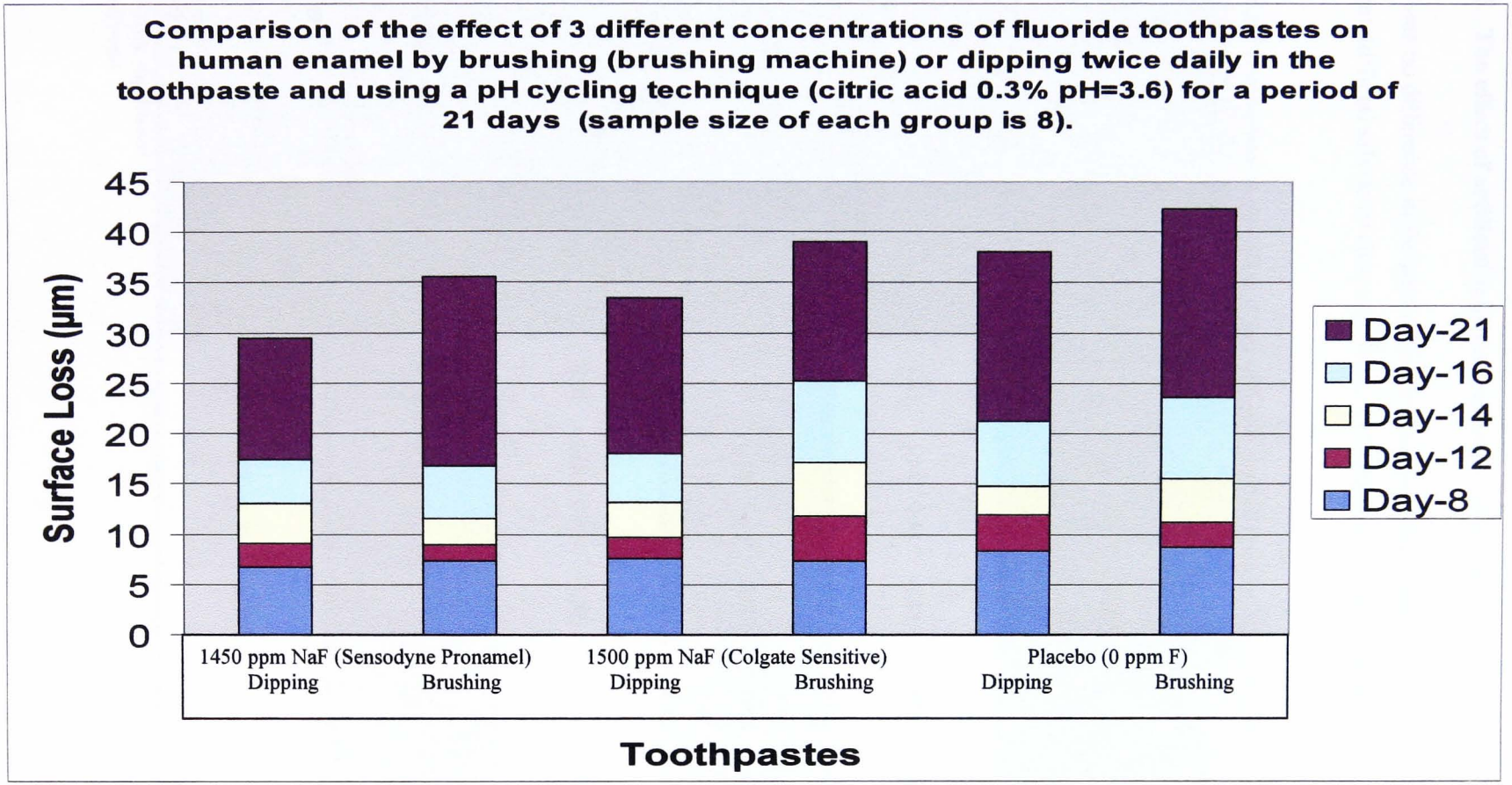
All toothpastes showed an increase in tooth surface loss in the groups receiving brushing at day 21 (Table 3.9). This increase of TSL was observed at an early stage with Colgate Sensitive but not with the Placebo or Sensodyne Pronamel (Figure 3.4).

Table 3.9: Comparison of the effect of brushing on human enamel erosion using three different concentrations of fluoride toothpastes using dipping with brushing (brushing machine) or dipping alone and a pH cycling technique (0.3% citric acid pH=3.6) for a period of 21 days (sample size of each group is 8).

Groups		Mean Diff (µm)	SD	SE	95% Confidence Interval of the Difference		Sig. (2-tailed)**
					Lower	Upper	
Sensodyne Pronamel (1450 ppm F)	Day-8: Dipping VS Brushing	-0.59	0.75	0.27	-1.21	0.04	0.06
	Day-12: Dipping VS Brushing	0.11	1.57	0.55	-1.20	1.43	0.84
	Day-14: Dipping VS Brushing	1.53	2.81	0.99	-0.82	3.88	0.17
	Day-16: Dipping VS Brushing	0.60	2.68	0.95	-1.63	2.84	0.54
	Day-21: Dipping VS Brushing	-6.11	5.33	1.89	-10.57	-1.65	0.01*
Colgate Sensitive (1500 ppm F)	Day-8: Dipping VS Brushing	0.25	0.88	0.31	-0.49	0.98	0.45
	Day-12: Dipping VS Brushing	-2.08	1.64	0.58	-3.45	-0.71	0.01*
	Day-14: Dipping VS Brushing	-3.82	1.58	0.56	-5.15	-2.50	0.00*
	Day-16: Dipping VS Brushing	-7.13	3.37	1.19	-9.94	-4.31	0.00*
	Day-21: Dipping VS Brushing	-5.62	4.54	1.61	-9.42	-1.83	0.01*
Placebo (0 ppm F)	Day-8: Dipping VS Brushing	-0.40	1.07	0.38	-1.30	0.50	0.33
	Day-12: Dipping VS Brushing	0.80	2.01	0.71	-0.88	2.48	0.30
	Day-14: Dipping VS Brushing	-0.87	2.18	0.77	-2.69	0.95	0.30
	Day-16: Dipping VS Brushing	-2.38	4.04	1.43	-5.76	1.00	0.14
	Day-21: Dipping VS Brushing	-4.18	6.72	2.38	-9.80	1.44	0.12

***Statistically significant**
**** paired t-test used to measure "*p*" values**

Figure 3.4: Comparison of the effect of three different concentrations of fluoride toothpastes on human enamel using dipping with brushing (brushing machine) or dipping alone twice daily in toothpastes and using a pH cycling technique (0.3% citric acid pH=3.6) for a period of 21 days (sample size of each group is 8).



3.3.4.2 The effect of artificial saliva on enamel erosion *in vitro*

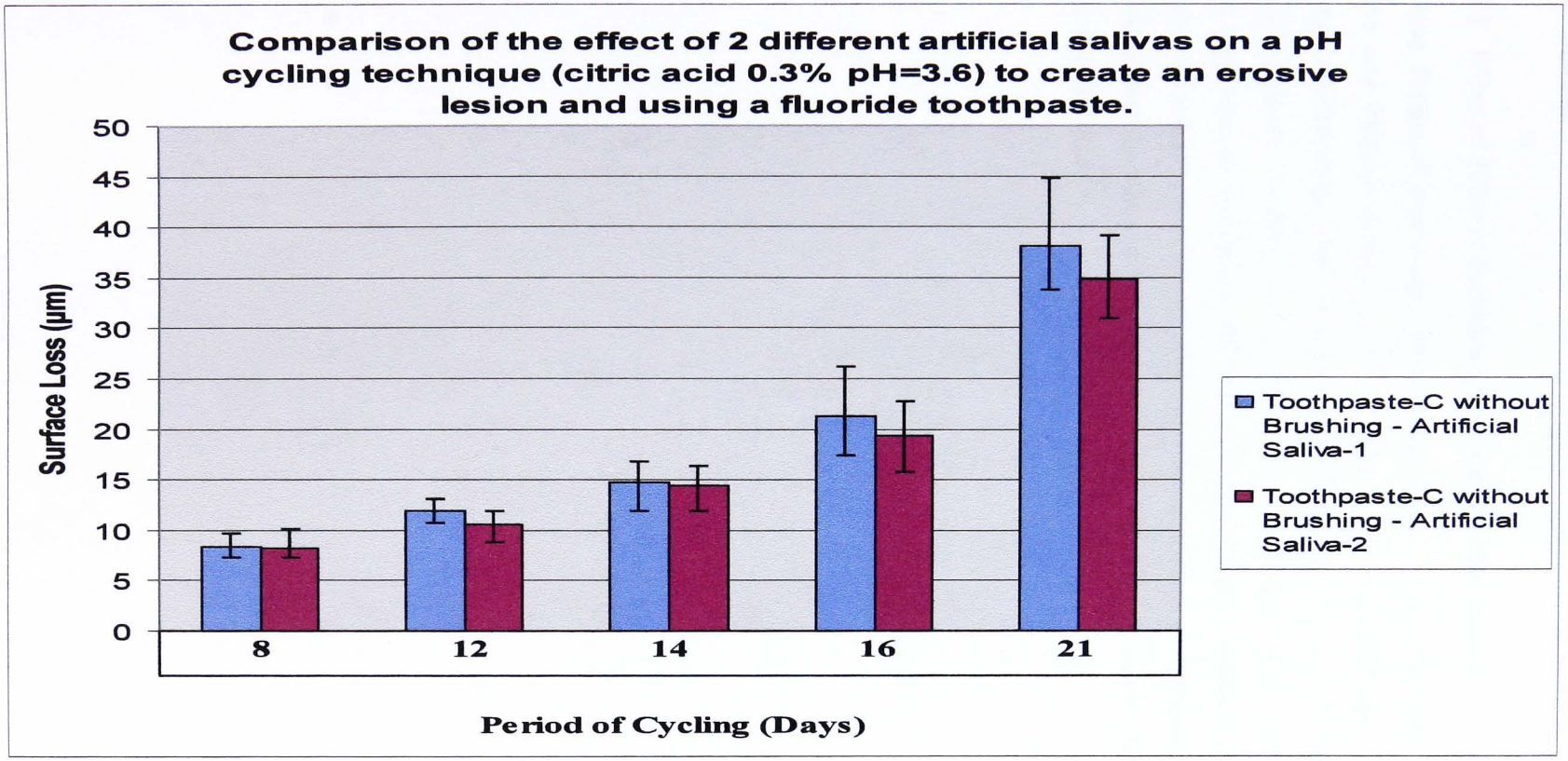
There was no difference in the amount of surface loss using 0ppm F toothpaste with two different artificial salvias as shown in Table 3.10, Figure 3.5, and Appendix 27.

Table 3.10: Comparison of the effect of two different artificial salivas on a pH cycling technique (0.3% citric acid pH=3.6) to create an erosive lesion and using a fluoride toothpaste.

Dipping Groups	Diff of Means (µm)	SD	SE	95% Confidence Interval of the Difference		Sig. (2-tailed)**
				Lower	Upper	
Day-8: Toothpaste-C (Artificial Saliva-1∫) VS Toothpaste-C (Artificial Saliva-2§)	0.21	1.54	0.54	-1.08	1.49	0.72
Day-12: Toothpaste-C (Artificial Saliva-1) VS Toothpaste-C (Artificial Saliva-2)	1.40	1.65	0.58	0.02	2.78	0.05
Day-14: Toothpaste-C (Artificial Saliva-1) VS Toothpaste- (Artificial Saliva-2)	0.31	2.01	0.71	-1.37	1.99	0.68
Day-16: Toothpaste-C (Artificial Saliva-1) VS Toothpaste-C (Artificial Saliva-2)	1.92	3.18	1.12	-0.74	4.58	0.13
Day-21: Toothpaste-C (Artificial Saliva-1) VS Toothpaste-C (Artificial Saliva-2)	3.29	5.59	1.98	-1.38	7.96	0.14

***Statistically significant**
****paired t-test**

Figure 3.5: Comparison of the effect of two different artificial saliva on a pH cycling technique (0.3% citric acid pH=3.6) to create an erosive lesion and using fluoride toothpaste (±SD)



3.3.4.3 Dentine

3.3.4.3.1 Effect of different fluoridated toothpastes on dentine erosion:

Sensodyne Pronamel toothpastes showed less tooth surface loss than both Colgate Sensitive and Placebo toothpastes at all time points in the groups when dipping, however, when brushing with toothpastes that was obvious at days 12, 14 and 21 only (Table 3.11, Figure 3.6, Table 3.12, Figure 3.7, and Appendix 28). At early stages up to day 12, there was no significant difference between Colgate Sensitive and Sensodyne Pronamel. After that time point the difference between these two groups increased. The statistical difference was not noted as in enamel. However, a similar trend of enamel erosion was observed.

Table 3.11: Comparison of the effect of three different concentrations of fluoride toothpastes on human dentine by dipping twice daily in the toothpaste and using a pH cycling technique (0.3% citric acid pH=3.6) for a period of 21 days (sample size of each group is 8).

Dipping Groups		Diff of Means (μm)	SD	SE	95% Confidence Interval of the Difference		Sig. (2-tailed) **
					Lower	Upper	
Day-8	Sensodyne Pronamel (1450 ppm F) VS Colgate Sensitive (1500 ppm F)	-0.22	1.39	0.49	-1.39	0.94	0.66
	Sensodyne Pronamel (1450 ppm F) VS Placebo	-2.20	1.05	0.37	-3.07	-1.32	0.00*
	Colgate Sensitive (1500 ppm F) VS Placebo	-1.97	1.35	0.48	-3.10	-0.84	0.00*
Day-12	Sensodyne Pronamel (1450 ppm F) VS Colgate Sensitive (1500 ppm F)	-0.28	2.40	0.85	-2.28	1.72	0.75
	Sensodyne Pronamel (1450 ppm F) VS Placebo	-2.54	1.64	0.58	-3.91	-1.17	0.00*
	Colgate Sensitive (1500 ppm F) VS Placebo	-2.26	2.15	0.76	-4.06	-0.46	0.02*
Day-14	Sensodyne Pronamel (1450 ppm F) VS Colgate Sensitive (1500 ppm F)	-3.30	3.34	1.18	-6.10	-0.51	0.03*
	Sensodyne Pronamel (1450 ppm F) VS Placebo	-4.64	2.92	1.03	-7.08	-2.19	0.00*
	Colgate Sensitive (1500 ppm F) VS Placebo	-1.33	2.48	0.88	-3.41	0.74	0.17
Day-16	Sensodyne Pronamel (1450 ppm F) VS Colgate Sensitive (1500 ppm F)	-1.11	3.23	1.14	-3.81	1.59	0.36
	Sensodyne Pronamel (1450 ppm F) VS Placebo	-3.64	5.01	1.77	-7.83	0.55	0.08
	Colgate Sensitive (1500 ppm F) VS Placebo	-2.53	3.85	1.36	-5.75	0.69	0.11
Day-21	Sensodyne Pronamel (1450 ppm F) VS Colgate Sensitive (1500 ppm F)	-4.14	6.50	2.30	-9.57	1.29	0.11
	Sensodyne Pronamel (1450 ppm F) VS Placebo	-5.71	6.99	2.47	-11.56	0.13	0.05*
	Colgate Sensitive (1500 ppm F) VS Placebo	-1.57	3.57	1.26	-4.55	1.41	0.25

***Statistically significant**

**** paired t-test used to measure "*p*" values**

Figure 3.6: Comparison of the effect of three different concentrations of fluoride toothpastes on human dentine using dipping twice daily in the toothpastes and using a pH cycling technique (0.3% citric acid, pH=3.6) for a period of 21 days (±SD).

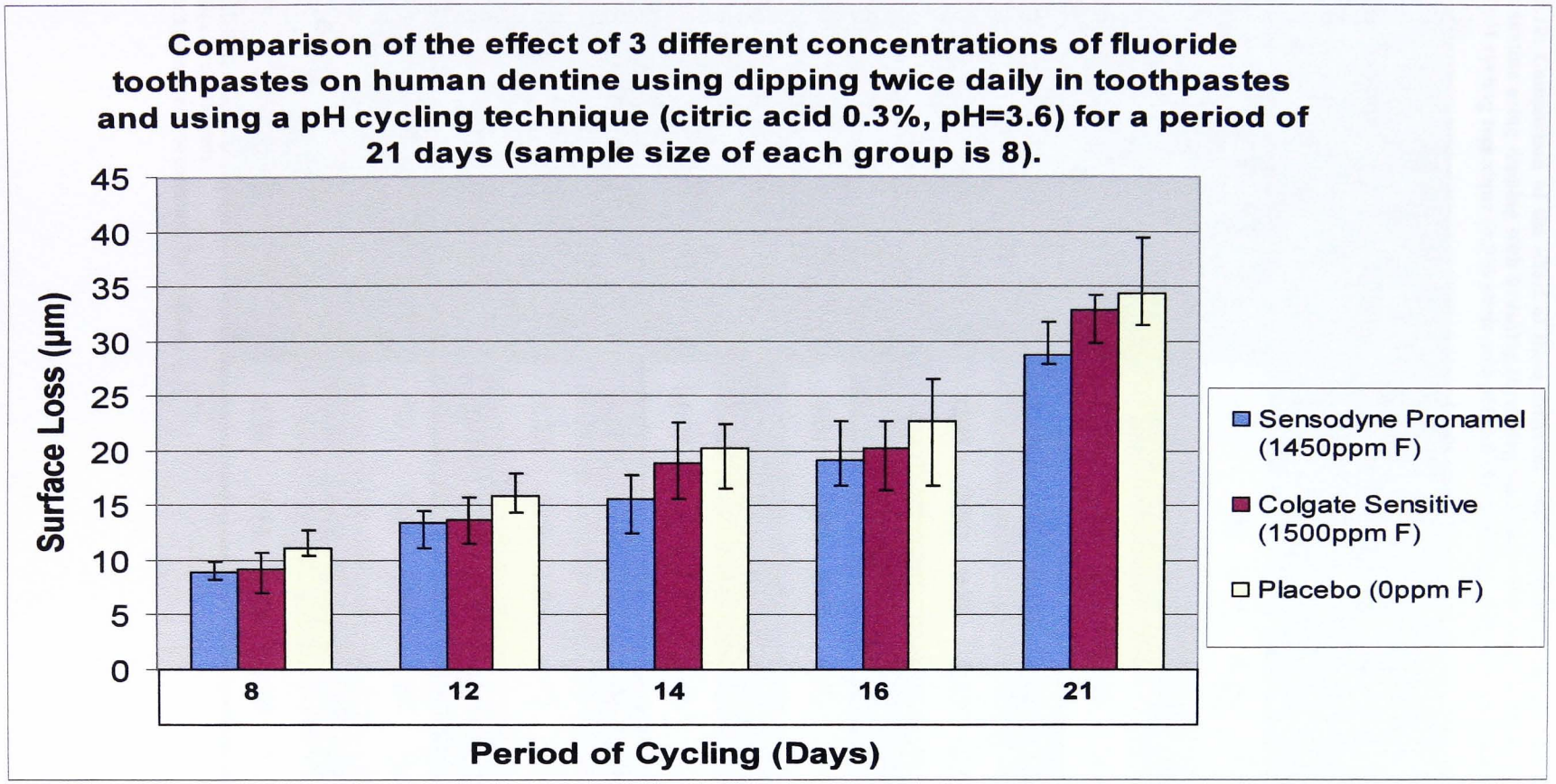
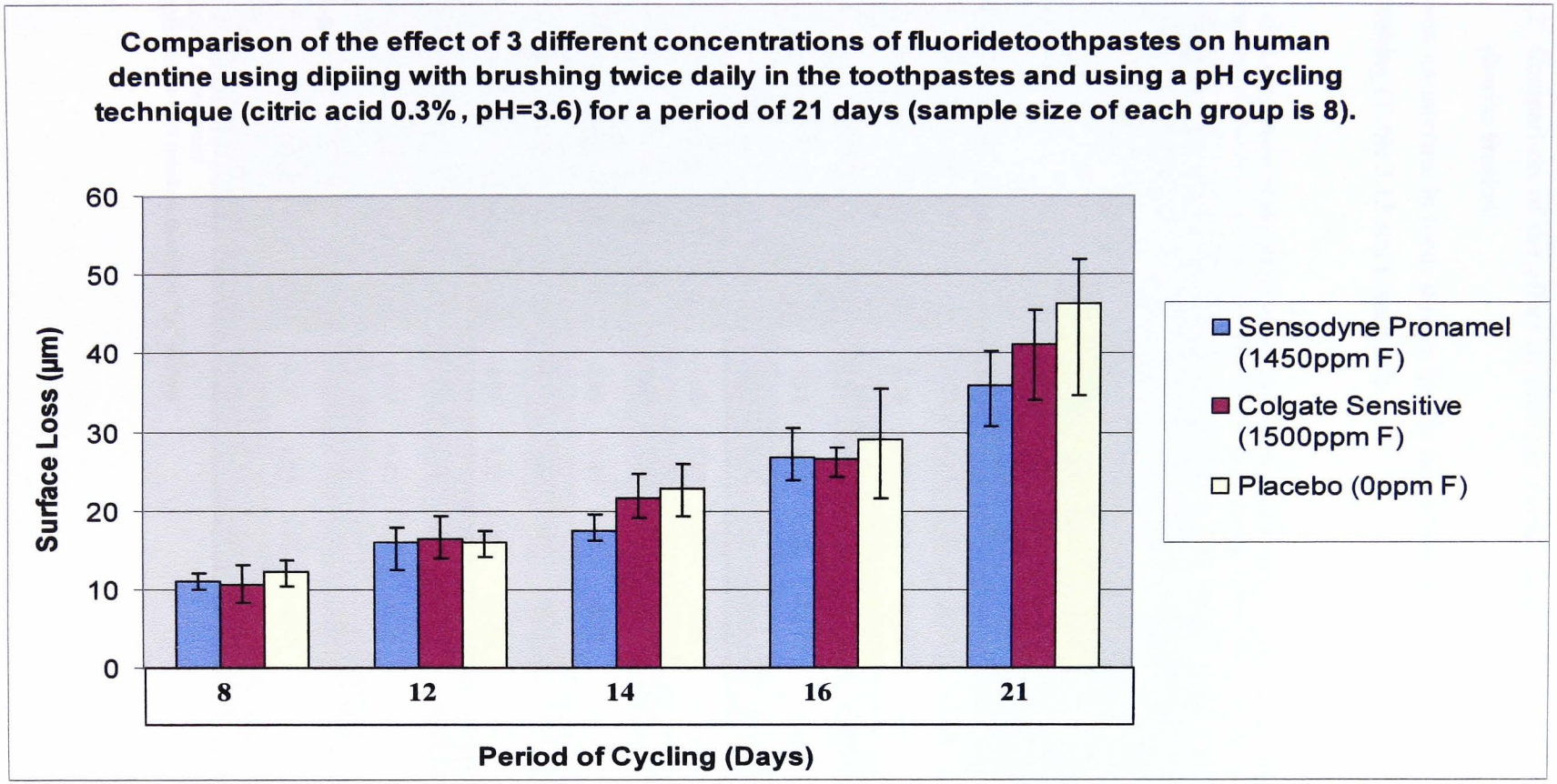


Table 3.12: Comparison of the effect of three different concentrations of fluoride toothpastes on human dentine using dipping with brushing (brushing machine) twice daily in the toothpaste and using a pH cycling technique (0.3% citric acid pH=3.6) for a period of 21 days (sample size of each group is 8).

Brushing Groups		Diff of Means (µm)	SD	SE	95% Confidence Interval of the Difference		Sig. (2-tailed) **
					Lower	Upper	
Day-8	Sensodyne Pronamel (1450 ppm F) VS Colgate Sensitive (1500 ppm F)	0.32	1.09	0.38	-0.59	1.23	0.43
	Sensodyne Pronamel (1450 ppm F) VS Placebo	-1.23	1.28	0.45	-2.30	-0.16	0.03*
	Colgate Sensitive (1500 ppm F) VS Placebo	-1.55	1.71	0.60	-2.98	-0.12	0.04*
Day-12	Sensodyne Pronamel (1450 ppm F) VS Colgate Sensitive (1500 ppm F)	-0.39	1.85	0.65	-1.94	1.16	0.57
	Sensodyne Pronamel (1450 ppm F) VS Placebo	-0.05	2.52	0.89	-2.15	2.06	0.96
	Colgate Sensitive (1500 ppm F) VS Placebo	0.34	2.02	0.71	-1.34	2.03	0.65
Day-14	Sensodyne Pronamel (1450 ppm F) VS Colgate Sensitive (1500 ppm F)	-4.01	1.80	0.64	-5.52	-2.51	0.00*
	Sensodyne Pronamel (1450 ppm F) VS Placebo	-5.24	2.43	0.86	-7.27	-3.21	0.00*
	Colgate Sensitive (1500 ppm F) VS Placebo	-1.23	2.88	1.02	-3.63	1.18	0.27
Day-16	Sensodyne Pronamel (1450 ppm F) VS Colgate Sensitive (1500 ppm F)	0.28	1.63	0.58	-1.08	1.64	0.64
	Sensodyne Pronamel (1450 ppm F) VS Placebo	-2.21	5.16	1.83	-6.53	2.11	0.27
	Colgate Sensitive (1500 ppm F) VS Placebo	-2.49	4.77	1.68	-6.47	1.49	0.18
Day-21	Sensodyne Pronamel (1450 ppm F) VS Colgate Sensitive (1500 ppm F)	-5.11	5.33	1.88	-9.57	-0.66	0.03*
	Sensodyne Pronamel (1450 ppm F) VS Placebo	-10.47	5.83	2.06	-15.34	-5.60	0.00*
	Colgate Sensitive (1500 ppm F) VS Placebo	-5.36	7.94	2.81	-12.00	1.28	0.10

***Statistically significant**
**** paired t-test used to measure "*p*" values**

Figure 3.7: Comparison of the effect of three different concentrations of fluoride toothpastes on human dentine by brushing twice daily in the toothpastes and using a pH cycling technique (0.3% citric acid, pH=3.6) for a period of 21 days (±SD).



3.3.4.3.2 Comparison of the effect of brushing versus dipping using toothpastes on dentine erosion:

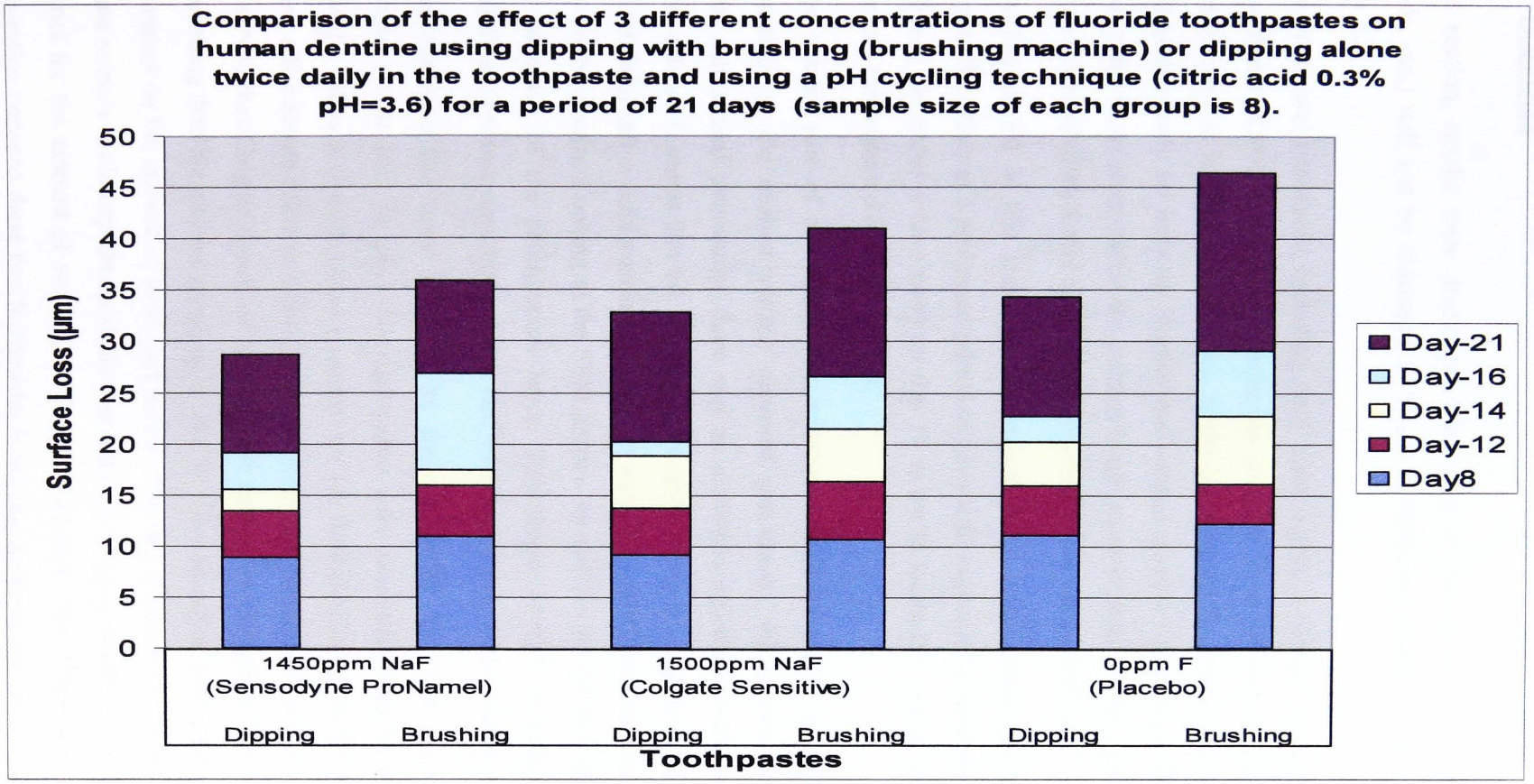
There was an increase in tooth surface loss in the groups when dipping was combined with brushing (Table 3.13 and Figure 3.8).

Table 3.13: Comparison of the effect of brushing on human dentine erosion using three different concentrations of fluoride toothpastes (dipping/brushing using a brushing machine) and a pH cycling technique (0.3% citric acid pH=3.6) for a period of 21 days (sample size of each group is 8).

		Mean Diff (µm)	SD	SE	95% Confidence Interval of the Difference		Sig. (2-tailed)**
					Lower	Upper	
Sensodyne Pronamel (1450 ppm F)	Day-8: dipping VS brushing	-2.02	0.57	0.20	-2.50	-1.54	0.00*
	Day-12: dipping VS brushing	-2.58	2.05	0.72	-4.29	-0.87	0.01*
	Day-14: dipping VS brushing	-1.96	2.23	0.79	-3.83	-0.10	0.04*
	Day-16: dipping VS brushing	-7.75	3.62	1.28	-10.78	-4.73	0.00*
	Day-21: dipping VS brushing	-7.23	7.20	2.55	-13.25	-1.21	0.03*
Colgate Sensitive (1500 ppm F)	Day-8: dipping VS brushing	-1.48	2.13	0.75	-3.26	0.30	0.09
	Day-12: dipping VS brushing	-2.69	2.59	0.92	-4.85	-0.52	0.02*
	Day-14: dipping VS brushing	-2.68	2.89	1.02	-5.09	-0.26	0.03*
	Day-16: dipping VS brushing	-6.36	2.83	1.00	-8.72	-4.00	0.00*
	Day-21: dipping VS brushing	-8.20	4.52	1.60	-11.98	-4.41	0.00*
Placebo (0 ppm F)	Day-8: dipping VS brushing	-1.06	1.35	0.48	-2.19	0.07	0.06
	Day-12: dipping VS brushing	-0.09	0.83	0.29	-0.78	0.61	0.78
	Day-14: dipping VS brushing	-2.57	2.72	0.96	-4.84	-0.30	0.03*
	Day-16: dipping VS brushing	-6.32	4.15	1.47	-9.79	-2.85	0.00*
	Day-21: dipping VS brushing	-11.99	5.23	1.85	-16.36	-7.61	0.00*

***Statistically significant**
**** Independent t-test used to measure "*p*" values**

Figure 3.8: Comparison of the effect of three different concentrations of fluoride toothpastes on human dentine using dipping with brushing (brushing machine) or dipping alone twice daily in the toothpaste and a using pH cycling technique (0.3% citric acid pH=3.6) for a period of 21 days (sample size of each group is 8).



3.3.5 Discussion

In this section, results were discussed. Other part of the study (i.e. study model, methods, etc.) will not be discussed as this was discussed earlier in chapter 2 and section 3.2.

In this study we introduced including only slabs within a certain range of Knoop microhardness (KMH) testing as an inclusion criterion in an attempt to eliminate the outliers we noticed in our previous work. KMH has reduced the amount of the outliers noticed in this study for both the dentine and enamel groups. The outliers which were noticed in this study were within the average range accepted statistically. Therefore, we would recommend using KMH as an inclusion criterion for future studies.

The cycling period in this study was increased to 21 days instead of 16 days to investigate the effect of a prolonged period of citric acid exposure on tooth surface loss. In addition, we added a time point on day 14 to try to locate the timing of the huge surface loss that occurred in the last study.

For the enamel part of this study, we introduced artificial saliva to minimise the precipitation on the enamel surface observed previously. When compared to the artificial saliva used previously, there was no statistical difference between the two artificial salivas. It seems that the amount of precipitation on the enamel surface caused by the artificial saliva used previously was very minimal and did not have an effective impact on the results. Looking at the SEM photos, we still see the enamel prisms even in the presence of the precipitation layer. This might provide evidence that the precipitation layer was very thin and thus had no effect on the surface loss of enamel.

Colgate Sensitive has more silica in its contents (RDA 83) than the other two toothpastes (RDA 40). The other two toothpastes were matched except for the amount of fluoride. Silica is considered as a competitor for fluoride binding to the surface. In addition, silica has an abrasive effect on hard oral tissues. Because of these two reasons, we observed that Colgate Sensitive caused more enamel tooth surface loss when used with brushing than the placebo group up to day 16. Then the effect of fluoride had more of an impact on the amount of tooth surface loss than the presence of the silica. Again, the same reasons could explain the difference between Colgate Sensitive and Sensodyne Pronamel for the amount of tooth surface loss of enamel. The difference of fluoride concentration between these two toothpastes is not big, however the amount of fluoride

release from Colgate Sensitive using our model is not similar to intra-oral scenario as Sodium monofluorophosphate (MFP) did not hydrolyse in our model. In addition, the amount of silica in Colgate Sensitive would explain the superiority of Sensodyne Pronamel regardless of the superiority of Colgate Sensitive in the concentration of fluoride.

For dentine, the scenario was different. The difference between toothpastes in the groups that received brushing was not noticed until day 21. Enamel and dentine have vastly different structures with dentine having a higher protein compared to enamel. This might explain the difference between the enamel and dentine. However, the difference between the groups that received dipping only and the groups that received brushing was similar to the trend seen in enamel.

The amount of tooth surface loss over a prolonged period of time in this study was less than observed in the previous study for enamel. The changes that happened by day 16 in the previous study were noticed with some slabs by day 16 but not on day 14. This change was delayed with some slabs until after day 16. Therefore, it is expected that the big change in tooth surface loss for enamel occurs around day 16, either just before or just after day 16. Less tooth surface loss in enamel in this study compared with the previous one was observed. The difference in the amount of tooth surface loss on enamel in this study and the previous study might be because of the interruption that happened during the cycling period and the storage of the slabs, or simply due to an error such as contamination of saliva with bacteria. However, the cycling technique we used had no sucrose. Therefore, the production of acids even in the presence of bacteria would not be expected. Another possibility is that the use of KMH as an inclusion criterion and the introduction of the enamel slabs in a narrow range of hardness had an impact.

The change of tooth surface loss with dentine over a prolonged period of pH cycling was investigated for the first time in this study. Therefore, it would be beneficial to repeat the study with dentine for reproducibility purposes.

In addition, the use of the new formulated artificial saliva showed no statistical difference in the amount of tooth surface loss in enamel. However, SEM showed that there was no precipitation on the enamel surface in this study. Therefore, it is recommended to use the new formulated artificial saliva.

Ganss *et al.*, (2007b) investigated the effectiveness of both waiting periods between acid exposure and tooth brushing and fluoride applications in preventing toothbrush abrasion of acid-softened enamel surfaces. The study, on five subjects, had an *in situ* crossover design with experimental periods of five days each. Human enamel samples were recessed in mouth appliances and at the end of each experimental period and enamel loss was determined profilometrically. Specimens were eroded extraorally (2 × 20 min per day; 0.05 M citric acid), standardized brushing (2 × 30 s per sample per day; powered toothbrush) was performed in situ. The groups were: (1) erosion only, (2) brushing with fluoride-free toothpaste directly after, (3) 2 h after, or (4) before erosion; fluoride application was either (5) brushing with a fluoride toothpaste or (6) brushing with a fluoride toothpaste or gel, and rinsing with a fluoride mouth rinse. Enamel loss was (1) 45.2±10.8, (2) 79.3±7.8, (3) 81.7±9.5, (4) 69.7±13.8, (5) 51.5±13.0, and (6) 41.2±1.8 µm. Brushing without fluoride increased the enamel loss significantly ($p \leq 0.001$), waiting for 2 h had no protective effect, and brushing before erosion decreased enamel loss values only by 12%. In the fluoride groups, enamel loss was significantly lower than after brushing with the fluoride-free toothpaste and comparable to values after erosion only. Waiting periods had only a minor effect, whilst the application of fluoride appeared promising.

Attin *et al.*, (2001b) demonstrated that pre-treatment fluoride delivery by a dentifrice, although beneficial against erosive loss (Davis and Winter, 1977), was not very effective in preventing erosive/abrasive lesions (Jaeggi and Lussi, 1999). A single toothbrushing for 30 sec on softened and sound enamel with different sequences of fluoride applications (Lussi *et al.*, 2004b) showed that the mean loss by brushing in situ with only water on sound enamel was 0.02-0.03 µm, whereas when brushing was undertaken on softened enamel with or without fluoride treatment the mean loss was 0.25-.27 µm and 0.20 µm respectively. In another in situ study (Hooper *et al.*, 2003) toothbrushing followed an orange juice drink four times daily and the mean enamel surface loss after 10 days ranged from 1.56 to 2.43 µm.

All of these studies demonstrated the increase surface loss when brushing was used. This was also evident in our model.

3.3.6 Conclusion

1. After 21 days cycling, Sensodyne Pronamel (1450 ppm NaF) showed significantly less tooth surface loss on enamel than the control and placebo groups without brushing on enamel, but this was not the case on dentine. Colgate Sensitive (1500 ppm NaF), showed no statitistical difference with placebo group. When brushing, Sensodyne Pronamel (1450 ppm NaF) showed significantly less tooth surface loss on enamel than the control and placebo groups on both enamel and dentine. Colgate Sensitive (1500 ppm NaF), showed no statitistical difference with placebo group. Therefore, the null hypothesis was rejected.
2. When compared abrasion effect (brushing) to non-abrasion (dipping) on enamel, there was statistical difference between them using fluoride toothpastes (1450 ppm NaF, and 1500 ppm NaF) but not in placebo group after our 21 days cycling model. However, the statistical difference was evident in all groups when dentine samples were used. Therefore, the second null hypothesis was rejected.
3. There was no difference between the two artificial salivas on the amount of tooth surface loss of enamel in this study. Therefore, the third null hypothesis was accepted.
4. Knoop microhardness (KMH) reduced the outliers observed in our statistical analysis. However, it was not possible check the significant level. Therefore, the acceptance or rejection of the null hypothesis cannot be confirmed. Due to the reduction of outliers, KMH was used as an inclusion criterion in the studies followed.

3.4 Study-6: The Effect of Different Concentrations of Sodium Fluoride Toothpaste on Tooth Surface Loss of the Dental Hard Tissues

3.4.1 Aims

To investigate the effects of five toothpastes with matched formulations, except in fluoride concentration, on enamel and dentine surface loss progression using an *in vitro* erosion cycling model for a period of 21 days.

The Null Hypothesis was that there is no difference in tooth surface loss of dental hard tissue with the increase of fluoride concentration in toothpastes.

3.4.2 Materials and Methods:

This study builds on a previous investigation which examined the effect of fluoride on human enamel for a period of 21 days. In this study, the cycling period was also 21 days and the experiment involved testing both enamel and dentine. In addition, KMH was used as one of the inclusion criteria in an attempt to eliminate the effect of the baseline difference of the slabs. The new formulation of artificial saliva was used in this study.

3.4.2.1 Slab Preparation

Five groups of eight human enamel slabs were cut from the buccal surface of intact human premolars. Slabs were mounted into resin blocks, ground and checked for surface flatness using a non-contact scanning profilometer (Scantron Proscan 2000). The slabs were checked for surface hardness using a micro-indenter measuring knoop microhardness (KMH). Five indents length were made in each slab surface. Slabs showing an average indent length between 60-70 μm for enamel slabs or 80-95 μm for dentine slabs were considered eligible for experiment inclusion. Thereafter, each surface was covered with nail varnish except for a small window (1x2 mm). The actual technique was described in section 3.2.2.1 (page 78).

3.4.2.2 Blindness

The five toothpastes used in the study were coded and the code was kept by the study sponsor. At the end of the study, the results were sent to the study sponsor and then the code was released to the study investigator.

3.4.2.3 Randomisation

Enamel slabs were randomly allocated to each study group using the toss of a coin.

3.4.2.4 Experimental Protocol/Regime

Each group in the study consisted of eight slabs of enamel/dentine. There were five groups of enamel and five groups of dentine in the study. A special tray with eight holes was used to hold the slabs which were embedded in resin blocks. The slabs were immersed under static conditions for two minutes five times daily in 0.3% citric acid (pH 3.6) for 21 days making a total exposure time of 210 minutes. In addition, the slabs were dipped twice daily in one of the test toothpastes for two minutes, once before cycling with citric acid and then after cycling. Each group of resin blocks were immersed in a 1:3 toothpaste slurry, morning and evening, for two minutes with one of five different toothpastes:

1. Toothpaste A: 0 ppm F (as NaF);
2. Toothpaste B: 250 ppm F (as NaF);
3. Toothpaste C: 500 ppm F (as NaF);
4. Toothpaste D: 1150 ppm F (as NaF);
5. Toothpaste E: 1450 ppm F (as NaF).

Toothpastes were prepared by adding three grams of toothpaste to 10 ml of de-ionised distilled water and mixed using a stirrer. During the cycling period, the slabs were analysed with the scanning profilometer to measure the amount of surface loss at days 7, 14 and 21.

3.4.2.5 Statistical Analysis

An SPSS statistical software package for Windows, version 13.0 (SPSS Inc., 1989-2002; LEAD technologies Inc., 1991-2000) was used for data analysis. Significance was

considered using "*p*" value ($p \leq 0.05$) and a confidence interval not including "ZERO" value when the groups were compared.

3.4.3 Results

3.4.3.1 Enamel

The enamel slabs for the five legs of the study were of different origin (i.e. different tooth) for each group.

Shapiro-Wilk test ($p \leq 0.05$) (Table 3.14) of the means of the enamel surface loss after treatment in all test groups showed that the results were normally distributed.

Table 3.14: Shapiro-Wilk test for measuring the normality of enamel surface loss.

	Shapiro-Wilk	
	df	Sig.
0 ppm F Day-7	8	0.957
0 ppm F Day-14	8	0.303
0 ppm F Day-21	8	0.128
250 ppm F Day-7	8	0.525
250 ppm F Day-14	8	0.487
250 ppm F Day-21	8	0.384
500 ppm F Day-7	8	0.241
500 ppm F Day-14	8	0.184
500 ppm F Day-21	8	0.047*
1150 ppm F Day-7	8	0.342
1150 ppm F Day-14	8	0.421
1150 ppm F Day-21	8	0.575
1450 ppm F Day-7	8	0.325
1450 ppm F Day-14	8	0.219
1450 ppm F Day-21	8	0.284

*** Significance**

The amount of tooth surface loss was significantly greater at day-7 when compared between toothpastes containing 0 ppm F and the higher fluoride concentration toothpastes (Table 3.15, Figure 3.9, and Appendix 31). This trend was more obvious at days-14 and 21 when compared with 1150 ppm F or 1450 ppm F groups only. This showed that there was a delay of the surface loss process with the low concentration fluoride toothpaste ($F \leq 500$ ppm) followed by an increase of tooth surface loss as the cycling period progressed.

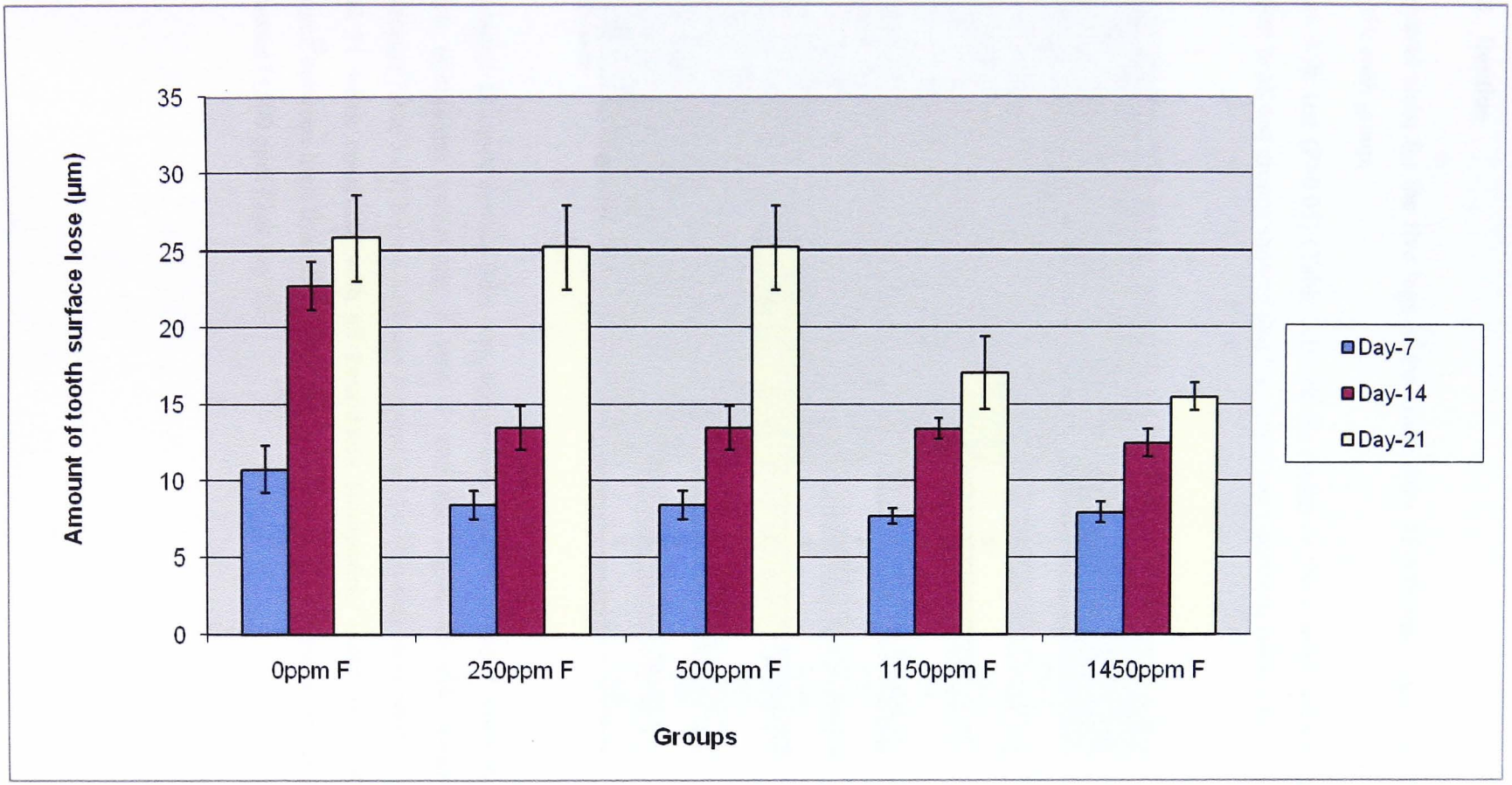
Table 3.15: Comparison of Mean Difference of Enamel Surface Loss after using 21 days cycling period and five matched toothpastes except in the amount of fluoride concentration as preventative agent.

Groups			Mean Diff	SD	95% Confidence Interval of the Difference		Sig. (2-tailed)**
					Lower	Upper	
7-Days	0 ppm F	VS 250 ppm F	2.323	0.997	0.928	3.717	0.004*
	0 ppm F	VS 500 ppm F	3.038	1.596	1.724	4.352	0.001*
	0 ppm F	VS 1150 ppm F	2.490	1.273	1.164	3.816	0.002*
	0 ppm F	VS 1450 ppm F	2.788	1.442	4.128	1.447	0.001*
	250 ppm	VS 500 ppm F	0.716	1.063	-0.092	1.523	0.077
	250 ppm	VS 1150 ppm F	0.168	0.722	-0.667	1.002	0.669
	250 ppm	VS 1450 ppm F	0.465	1.062	1.334	-0.404	0.268
	500 ppm F	VS 1150 ppm F	0.548	0.693	1.124	-0.028	0.061
	500 ppm F	VS 1450 ppm F	0.250	0.682	0.389	-0.889	0.412
	1150 ppm F	VS 1450 ppm F	0.298	0.758	0.976	-0.381	0.362
14-Days	0 ppm F	VS 250 ppm F	9.291	2.298	7.669	10.914	0.000*
	0 ppm F	VS 500 ppm F	9.354	2.057	7.991	10.717	0.000*
	0 ppm F	VS 1150 ppm F	10.615	2.576	8.801	12.428	0.000*
	0 ppm F	VS 1450 ppm F	10.278	2.016	11.687	8.870	0.000*
	250 ppm	VS 500 ppm F	0.063	1.292	-1.200	1.326	0.914
	250 ppm	VS 1150 ppm F	1.323	2.772	-0.433	3.079	0.128
	250 ppm	VS 1450 ppm F	0.987	1.565	-2.302	0.328	0.128
	500 ppm F	VS 1150 ppm F	1.260	1.928	-0.278	2.798	0.097
	500 ppm F	VS 1450 ppm F	0.924	0.904	-1.788	-0.060	0.038*
	1150 ppm F	VS 1450 ppm F	0.336	1.674	-1.238	1.911	0.645
21-Days	0 ppm F	VS 250 ppm F	0.657	3.896	-2.282	3.596	0.639
	0 ppm F	VS 500 ppm F	8.756	4.741	5.969	11.543	0.063
	0 ppm F	VS 1150 ppm F	2.775	1.908	-0.172	5.722	0.000*
	0 ppm F	VS 1450 ppm F	10.335	3.271	-12.694	-7.976	0.000*
	250 ppm	VS 500 ppm F	8.100	4.341	5.359	10.840	0.140
	250 ppm	VS 1150 ppm F	2.119	4.411	-0.786	5.023	0.000*
	250 ppm	VS 1450 ppm F	9.678	2.634	-11.973	-7.384	0.000*
	500 ppm F	VS 1150 ppm F	5.981	4.046	-8.730	-3.232	0.000*
	500 ppm F	VS 1450 ppm F	1.579	2.553	-3.625	0.467	0.000*
	1150 ppm F	VS 1450 ppm F	7.560	3.535	-9.866	-5.253	0.115

***Significance**

**** paired t-test used to measure "*p*" values**

Figure 3.9: Comparison of Mean Difference of Enamel Surface Loss after using 21 days cycling period and five matched toothpastes except for the amount of fluoride concentration as preventative agent.



3.4.3.2 Dentine

The enamel slabs for the five legs of the study were of different origin (i.e. different tooth) for each group.

Shapiro-Wilk test (P>0.05) (Table 3.16) of the means of the enamel surface loss after treatment in all test groups showed that the results were normally distributed.

Table 3.16: Shapiro-Wilk test for measuring the normality of dentine surface loss.

	Shapiro-Wilk	
	df	Sig.
0 ppm F Day-7	8	0.020
0 ppm F Day-14	8	0.516
0 ppm F Day-21	8	0.665
250 ppm F Day-7	8	0.716
250 ppm F Day-14	8	0.191
250 ppm F Day-21	8	0.263
500 ppm F Day-7	8	0.653
500 ppm F Day-14	8	0.216
500 ppm F Day-21	8	0.180
1150 ppm F Day-7	8	0.345
1150 ppm F Day-14	8	0.500
1150 ppm F Day-21	8	0.455
1450 ppm F Day-7	8	0.483
1450 ppm F Day-14	8	0.173
1450 ppm F Day-21	8	0.180

*** Significance**

The amount of tooth surface loss was significantly greater at day-7 when compared between toothpastes containing 0 ppm F and the higher fluoride concentration toothpastes (Table 3.17, Figure 3.10 and Appendix 32). This was more obvious at days-14 and 21 when compared with all fluoridated toothpastes. There was a statistical difference between high fluoride toothpastes (≥1150 ppm F) compared to low fluoride toothpastes (≤500 ppm F) at day 21.

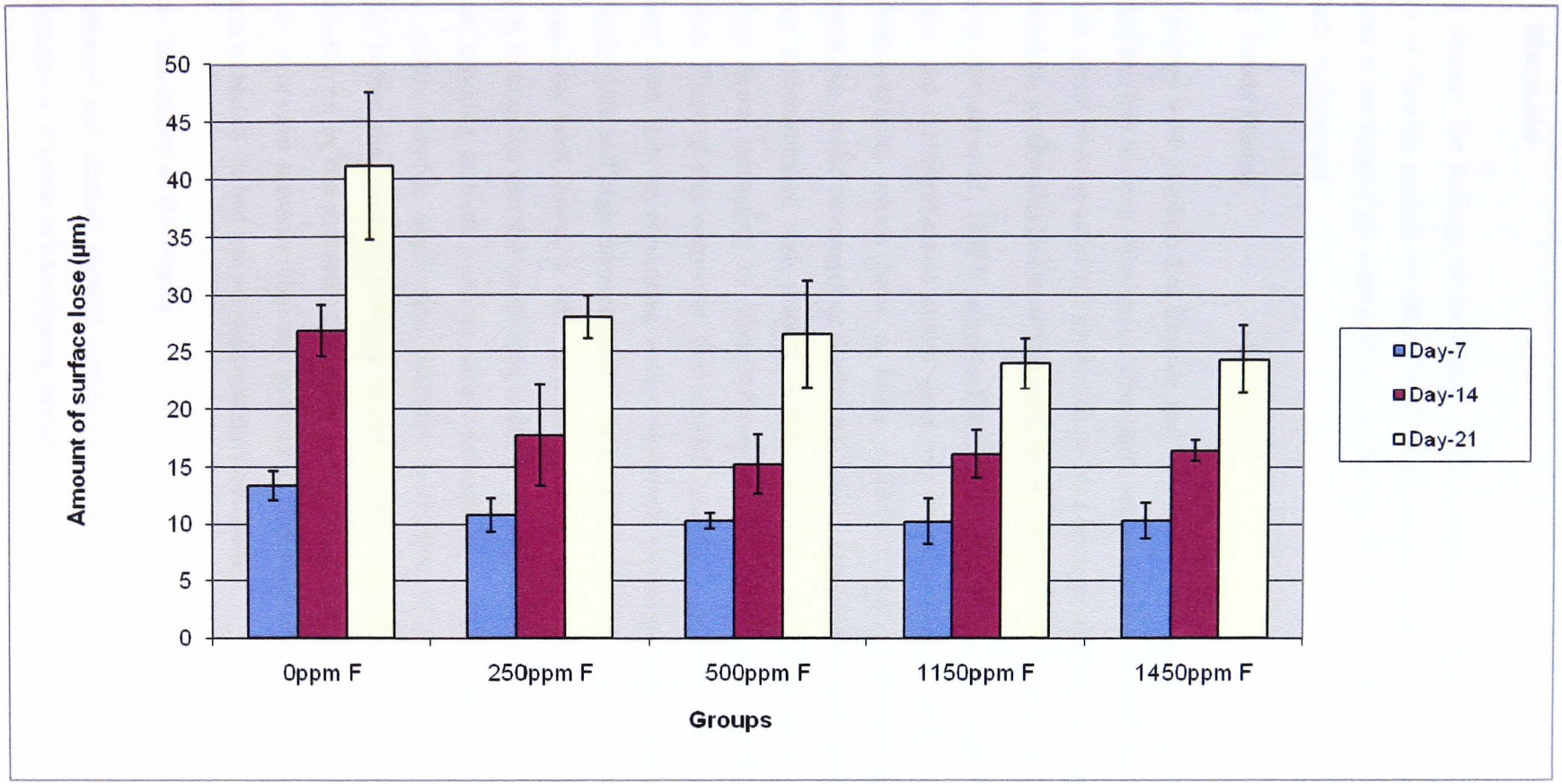
Table 3.17: Comparison of Mean Difference of Dentine Surface Loss after using 21 days cycling period and five matched toothpastes except in the amount of fluoride concentration as preventative agent.

Groups			Mean Diff	SD	95% Confidence Interval of the		Sig. (2-tailed)**
					Lower	Uppe	
7-Days	0 ppm F	VS 250 ppm F	2.53	0.69	1.06	4.00	0.01*
	0 ppm F	VS 500 ppm F	3.08	0.51	1.98	4.18	0.00*
	0 ppm F	VS 1150 ppm F	3.12	0.83	1.34	4.89	0.00*
	0 ppm F	VS 1450 ppm F	3.07	0.71	-4.60	-1.55	0.00*
	250 ppm	VS 500 ppm F	0.55	0.57	-0.67	1.77	0.34
	250 ppm	VS 1150 ppm F	0.59	0.86	-1.26	2.44	0.67
	250 ppm	VS 1450 ppm F	0.55	0.75	-2.16	1.06	0.40
	500 ppm F	VS 1150 ppm F	0.04	0.73	-1.61	1.53	0.60
	500 ppm F	VS 1450 ppm F	0.00	0.60	-1.28	1.28	0.53
	1150 ppm F	VS 1450 ppm F	0.04	0.88	-1.85	1.93	0.53
14-Days	0 ppm F	VS 250 ppm F	9.10	1.75	5.34	12.86	0.00*
	0 ppm F	VS 500 ppm F	11.59	1.21	8.98	14.19	0.00*
	0 ppm F	VS 1150 ppm F	1.61	1.73	-2.11	5.33	0.21
	0 ppm F	VS 1450 ppm F	10.41	0.85	-12.24	-8.59	0.00*
	250 ppm	VS 500 ppm F	2.49	1.82	-1.41	6.39	0.14
	250 ppm	VS 1150 ppm F	1.61	1.73	-2.11	5.33	0.21
	250 ppm	VS 1450 ppm F	1.32	1.60	-4.75	2.11	0.14
	500 ppm F	VS 1150 ppm F	0.88	1.19	-1.66	3.42	0.67
	500 ppm F	VS 1450 ppm F	1.17	0.98	-0.93	3.27	0.67
	1150 ppm F	VS 1450 ppm F	0.29	0.81	-1.45	2.03	1.00
21-Days	0 ppm F	VS 250 ppm F	13.18	2.36	8.12	18.23	0.00*
	0 ppm F	VS 500 ppm F	14.65	2.80	8.65	20.64	0.00*
	0 ppm F	VS 1150 ppm F	17.16	2.39	12.04	22.29	0.00*
	0 ppm F	VS 1450 ppm F	16.81	2.49	-22.15	-11.47	0.00*
	250 ppm	VS 500 ppm F	1.47	1.77	-2.32	5.25	0.92*
	250 ppm	VS 1150 ppm F	3.99	1.00	1.83	6.14	0.00*
	250 ppm	VS 1450 ppm F	3.64	1.22	-6.26	-1.02	0.02*
	500 ppm F	VS 1150 ppm F	2.52	1.81	-6.40	1.37	0.07*
	500 ppm F	VS 1450 ppm F	2.17	1.94	-6.33	1.99	0.17
	1150 ppm F	VS 1450 ppm F	-0.35	1.29	-2.41	3.11	0.60

***Significance**

**** paired t-test used to measure "*p*" values**

Figure 3.10: Comparison of Mean Difference of Dentine Surface Loss after using 21 days cycling period and five matched toothpastes except in the amount of fluoride concentration as preventative agent.



3.4.4 Discussion

In this section, the findings of the effects of five matched toothpastes except for the amount of fluoride content on dental hard tissue erosion under erosive conditions is discussed in the light of the current literature. Moreover, the methodology employed in this study is discussed.

3.4.4.1 Study Design

Our previous work showed that fluoride had an effect in reduction of the amount of tooth surface loss *in vitro*. Previous *in vitro* work suggested that fluoride had an impact effect on dental erosion as an ion present in saliva (Amaechi and Higham, 2001) or as compound i.e. toothpastes and mouthrinse (Kelly and Smith, 1988; Attin *et al.*, 2003).

However, Sorvari *et al.*, (1994) suggested that fluoride present in the mouth during the daily de- and remineralisation cycles gave rise to the formation of fluorapatite or fluorhydroxyapatite, which have a lower solubility than hydroxyapatite. Acidic challenges (i.e. acidic beverages or foodstuffs) have a composition and a pH such that they are undersaturated with respect to these minerals and consequently even the outermost layer consisting of fluor(hydroxy)apatite will dissolve. Therefore, the protective effect of this outermost fluoride-rich mineral in preventing erosion is less important than it is in preventing caries. However, treatment with fluoride varnish (2.26%) for 24 h and high concentration F rinses (1.2%) for 48 h applied prior to acidic challenge have been shown to offer *in vitro* protection against erosion (Sorvari *et al.*, 1994). It is assumed that this protection is due to precipitation of calcium fluoride-like particles adhering to tooth surfaces which subsequently released fluoride over time. Hence, gentle fluoride application (without destruction of the protective acquired pellicle) before the erosive challenge would be most beneficial. This is what was suggested in an in situ scenario. Therefore, it was deemed necessary to examine this effect in an *in vitro* scenario. This was particularly important after we developed our *in vitro* model and as we had not yet used matched toothpastes.

3.4.4.2 Discussion of the results

Both enamel and dentine showed significantly reduced surface loss with increased concentration of fluoride in a toothpaste formulation.

The results observed on tooth surface loss of enamel between toothpastes showed that toothpastes with a fluoride concentration over 500 ppm F significantly decreased the amount of erosion.

This supports the previous findings indicating that fluoridated toothpastes can offer protection to enamel from erosive challenges (Fowler *et al.*, 2006; Zero *et al.*, 2006).

Fowler *et al.* (2006) in an enamel surface softening study, human enamel was pre-treated with one of four toothpaste slurries (new test formulation fluoride toothpaste, Elmex Sensitive, Colgate Sensitive, and placebo) for two minutes, before exposure to 1.0% citric acid, pH 3.8, for a total of 30 min. The surface microhardness (SMH) of the specimens was determined at baseline and at 10-minute intervals using a Struers Duramin-1 microindentor. Lesion repair was monitored by SMH after 4, 24, and 48 h incubation in artificial saliva. This remineralising phase was modified by the addition of an aliquot of the relevant toothpaste slurry, to mimic in vivo carryover of the formulation. The new test formulation, Elmex Sensitive, and Colgate Sensitive exhibited statistically significant inhibition of citric acid-mediated enamel surface softening versus a fluoride-free placebo at all time points. The test toothpaste gave statistically superior protection against the erosive challenge compared to Elmex Sensitive and Colgate Sensitive after 20- and 30-minute exposures.

Zero *et al.*, (2006) conducted an *in situ* double-blind crossover design study with three phases using six adult volunteers. Each subject wore a palatal device contained six human dental enamel slabs. The slabs were previously demineralised by 1.0% citric acid, pH 3.8. Each subject had to dip the device three times a day in one of the following treatments: non-fluoridated dentifrice (negative control); dentifrice containing 1,100 μg F/g, pH 7.0 (positive control); dentifrice containing 550 μg F/g, pH 5.5 (experimental). At the end of each phase, enamel remineralisation was assessed in terms of cross-sectional microhardness, and loosely as well as firmly bound fluoride formation was determined on the enamel surface. Fluoridated dentifrices were more effective than the negative control in forming loosely and firmly bound fluoride on enamel ($p<0.05$). However, the positive control formed more loosely bound fluoride than the other treatments ($p<0.05$). Microhardness analysis showed that the fluoridated dentifrices were more effective than the negative control ($p<0.05$) in remineralising dental enamel, although no statistically significant difference was observed between them. Thus, the experimental dentifrice was shown to be effective in remineralising dental enamel. The

researchers assumed that this may be due to its ability to form firmly bound fluoride on enamel.

The results observed on tooth surface loss of dentine between toothpastes showed that toothpastes with a fluoride concentration equal or above 250 ppm F significantly decreased the amount of erosion.

Magalhães *et al.* (2008a) in an *in situ/ex vivo* study assessed the effect of different concentrations of fluoride in dentifrices on dentine subjected to erosion or to erosion plus abrasion. Ten volunteers took part in this crossover and double-blind study performed in three phases (seven days). They wore acrylic palatal appliances containing four bovine dentine blocks divided into two rows: erosion and erosion plus abrasion. The blocks were subjected to erosion by immersion *ex vivo* in a cola drink (60 s, pH 2.6) four times daily. During this step, the volunteers brushed their teeth with one of three dentifrices D (5,000 ppm F, NaF, silica); C (1,100 ppm F, NaF, silica) and placebo (22 ppm F, silica). Then, the respective dentifrice slurry (1:3) was dripped onto the dentine surfaces. While no further treatment was performed in one row, the other row was brushed using an electric toothbrush for 30 s *ex vivo*. The appliances were replaced in the mouth and the volunteers rinsed with water. Dentine loss was determined by profilometry and analyzed using a two way ANOVA/Bonferroni test (a=0.05). Dentine loss after erosive-abrasive wear was significantly greater than after erosion alone. Wear was significantly higher for the placebo than for D and C dentifrices, which were not significantly different from each other. It can be concluded that the presence of fluoride concentrations around 1,100 ppm F in dentifrices is important to reduce dentine wear by erosion and erosion plus abrasion, but the protective effect does not increase with fluoride concentration.

However this was an *in situ* model and bovine dentine was used, it gives an indication that the effect of fluoride alone (i.e. without abrasion) reduced the amount of tooth surface loss which was also confirmed by our findings.

3.4.5 Conclusion

In this 21-day *in vitro*, erosion cycling model, all other toothpastes parameter being equal, a fluoride dose-response was observed in enamel and dentine surface loss progression after twice daily treatment with a toothpaste. The null hypothesis was rejected in this study.

3.5 Study-7: The Effect of Different Levels of Abrasives in Toothpaste on Tooth Surface Loss of the Dental Hard Tissues

3.5.1 Aims

To investigate the effects of three toothpastes with matched formulations, except for abrasive content, on enamel and dentine surface loss progression using an *in vitro* erosion cycling model for a period of 21 days.

Therefore, the null Hypothesis was that there is no difference between different RDA contents on tooth surface loss of dental hard tissues.

3.5.2 Materials and Methods:

This study builds on a previous investigation which examined the effect of fluoride on human enamel for a period of 21 days. In this study, the cycling period was also 21 days and the experiment involved testing both enamel and dentine. In addition, KMH was used as one of the inclusion criteria in an attempt to eliminate the effect of the baseline difference of slabs used. The new formulation of artificial saliva was used in this study.

3.5.2.1 Slab Preparation

Three groups of eight human enamel slabs were cut from the buccal surface of intact human premolars. The technique is fully described in sections 2.4.1 (page 25) and 3.4.2.1(page 103).

3.5.2.2 Blindness

The five toothpastes used in the study were coded and the code was kept by the study sponsor. At the end of the study, the results were sent to the study sponsor and then the code was released to the study investigator.

3.5.2.3 Randomisation

Enamel slabs were randomly allocated to each study group using the toss of a coin.

3.5.2.4 Experimental Protocol/Regime

Each group in the study consisted of eight slabs of enamel/dentine. There were three groups of enamel and three groups of dentine in the study.

A special tray with eight holes was used to hold the slabs which were embedded in resin blocks. The slabs were immersed under static conditions for two minutes five times daily in 0.3% citric acid (pH 3.6) for 21 days making a total exposure time of 210 minutes. In addition, the slabs were dipped twice daily in one of the test toothpastes for two minutes, once before cycling with citric acid and then after cycling. Each group of resin blocks were immersed in a 1:3 toothpaste slurry, morning and evening, for two minutes with one of three different toothpastes:

1. Toothpaste X: 140 RDA;
2. Toothpaste Y: 160 RDA;
3. Toothpaste Z: 40 RDA.

In addition, toothpastes contained 1450 ppm F. Toothpastes were prepared by adding three grams of toothpaste to 10 ml of de-ionised distilled water and mixed using a stirrer.

Toothbrushes of medium coarse bristles (Sainsbury's, Sainsbury's Supermarket Ltd, London, Produced in China) were used. Fifteen strokes of 200 g weight were applied using a brushing machine (NEL-BSI Dentifrice Test Machine, UK) for the groups received brushing during dipping in the toothpastes.

During the cycling period, the slabs were analysed with the scanning profilometer to measure the amount of surface loss at days 7, 14 and 21.

3.5.2.5 Statistical Analysis

An SPSS statistical software package for Windows, version 13.0 (SPSS Inc., 1989-2002; LEAD technologies Inc., 1991-2000) was used for data analysis. Significance was considered using "*p*" value ($p \leq 0.05$) and a confidence interval not including "ZERO" value when the groups were compared.

3.5.3 Results

3.5.3.1 Enamel

The enamel slabs for the five legs of the study were of different origin (i.e. different tooth) for each group.

Shapiro-Wilk test ($p \leq 0.05$) (Table 3.18) of the means of the enamel surface loss after treatment in all test groups showed that the results were normally distributed.

Table 3.18: Shapiro-Wilk test for measuring the normality of enamel surface loss.

	Shapiro-Wilk	
	df	Sig.
RDA 140 Day-7	8	0.553
RDA 140 Day-14	8	0.209
RDA 140 Day-21	8	0.176
RDA 160 Day-7	8	0.480
RDA 160 Day-14	8	0.771
RDA 160 Day-21	8	0.761
RDA 40 Day-7	8	0.900
RDA 40 Day-14	8	0.238
RDA 40 Day-21	8	0.058

*** Significance**

Paired sample t-test was used for calculating the confidence intervals *(Equal variances not assumed).*

The amount of tooth surface loss was significantly less at day-7 when comparing toothpaste containing 40 RDA with the higher RDA content (Table 3.19, Figure 3.11, and Appendix 33). This trend was not observed at days 14 and 21.

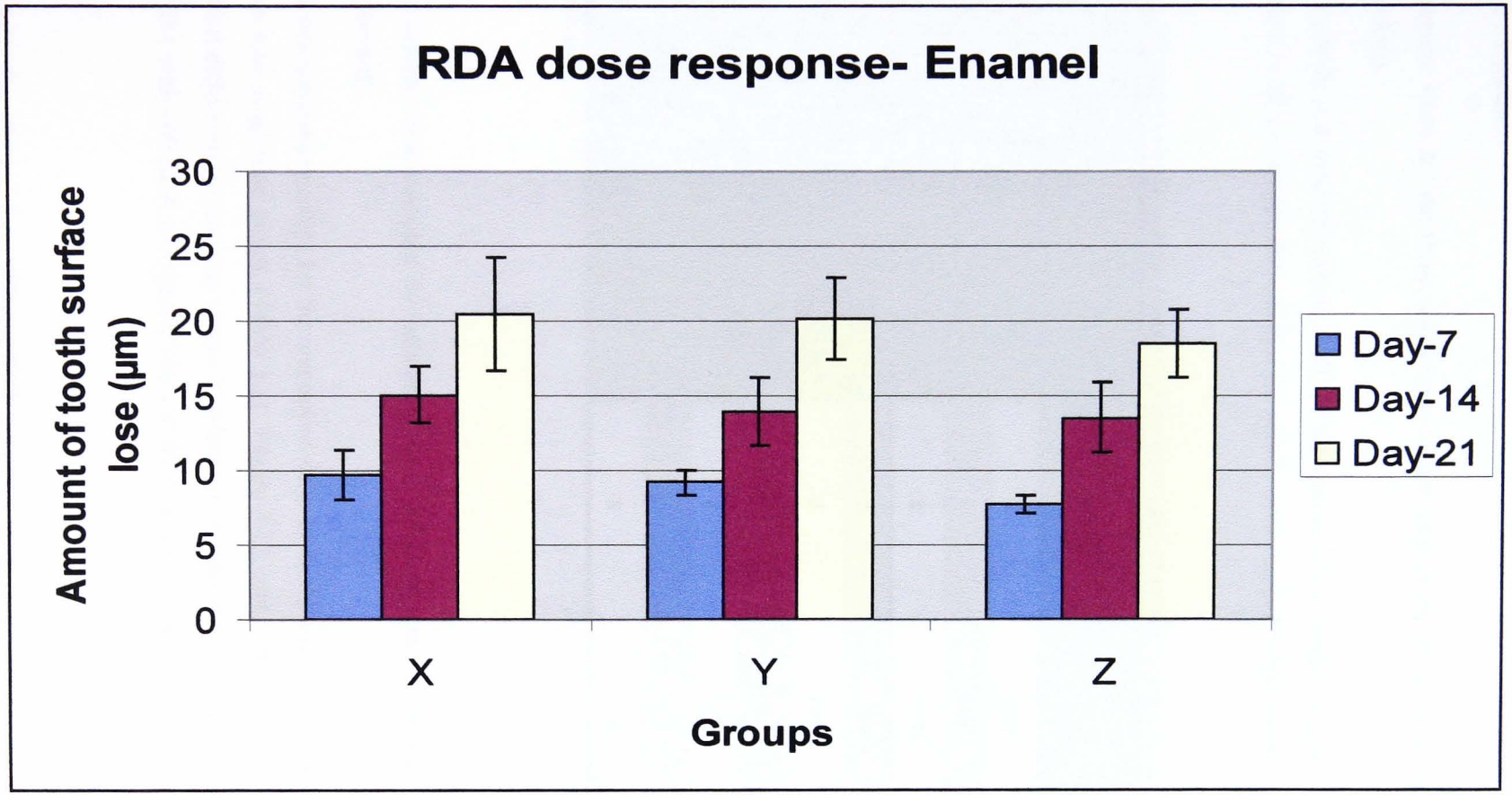
Table 3.19: The effect of three different RDA content in toothpastes on the amount of enamel surface loss caused by using a 21-day pH cycling technique.

Days	Groups	Mean Diff (µm)	SD (µm)	Confidence Interval (95%) **		Sig.** (2-tailed)
				min	max	
Day - 7	RDA 140 VS RDA 160	0.491	2.047	-1.220	2.202	0.519
	RDA 140 VS RDA 40	1.951	1.960	0.312	3.589	0.026*
	RDA 160 VS RDA 40	1.460	1.055	0.577	2.342	0.006*
Day - 14	RDA 140 VS RDA 160	1.089	3.258	-1.635	3.812	0.376
	RDA 140 VS RDA 40	1.518	2.849	-0.864	3.900	0.175
	RDA 160 VS RDA 40	0.429	3.954	-2.876	3.735	0.768
Day - 21	RDA 140 VS RDA 160	0.283	2.589	-1.882	2.447	0.766
	RDA 140 VS RDA 40	1.895	4.544	-1.904	5.695	0.277
	RDA 160 VS RDA 40	1.613	4.090	-1.807	5.032	0.302

*** Significance**

**** Paired sample t-test was used for calculating the confidence intervals**

Figure 3.11: The effect of three different RDA content in toothpastes on the amount of enamel surface loss caused by using a 21-day pH cycling technique. (Toothpaste X: 140 RDA; Toothpaste Y: 160 RDA; and Toothpaste Z: 40 RDA).



3.5.3.2 Dentine

The dentine slabs for the three test groups of the study were of the same origin (i.e. same tooth).

Shapiro-Wilk test ($p \leq 0.05$) (Table 3.20) of the means of the enamel surface loss after treatment in all test groups showed that the results were normally distributed.

Table 3.20: Shapiro-Wilk test for measuring the normality of enamel surface loss.

	Shapiro-Wilk	
	df	**Sig.**
RDA 140 Day-7	8	0.168
RDA 140 Day-14	8	0.853
RDA 140 Day-21	8	0.732
RDA 160 Day-7	8	0.196
RDA 160 Day-14	8	0.468
RDA 160 Day-21	8	0.281
RDA 40 Day-7	8	0.356
RDA 40 Day-14	8	0.052
RDA 40 Day-21	8	0.229

*** Significance**

Paired sample t-test was used for calculating the confidence intervals *(Equal variances not assumed).*

It was not noticed any effect for the amount of silica in toothpaste on reduction of tooth surface loss at all time points (Table 3.21, Figure 3.12, and Appendix 34). The only statistical difference noticed was at day-21 when we compared the toothpaste containing 140 RDA with 160 RDA toothpaste. The 160 RDA toothpaste showed less tooth surface loss.

Table 3.21: The effect of three different RDA content in toothpastes on the amount of enamel

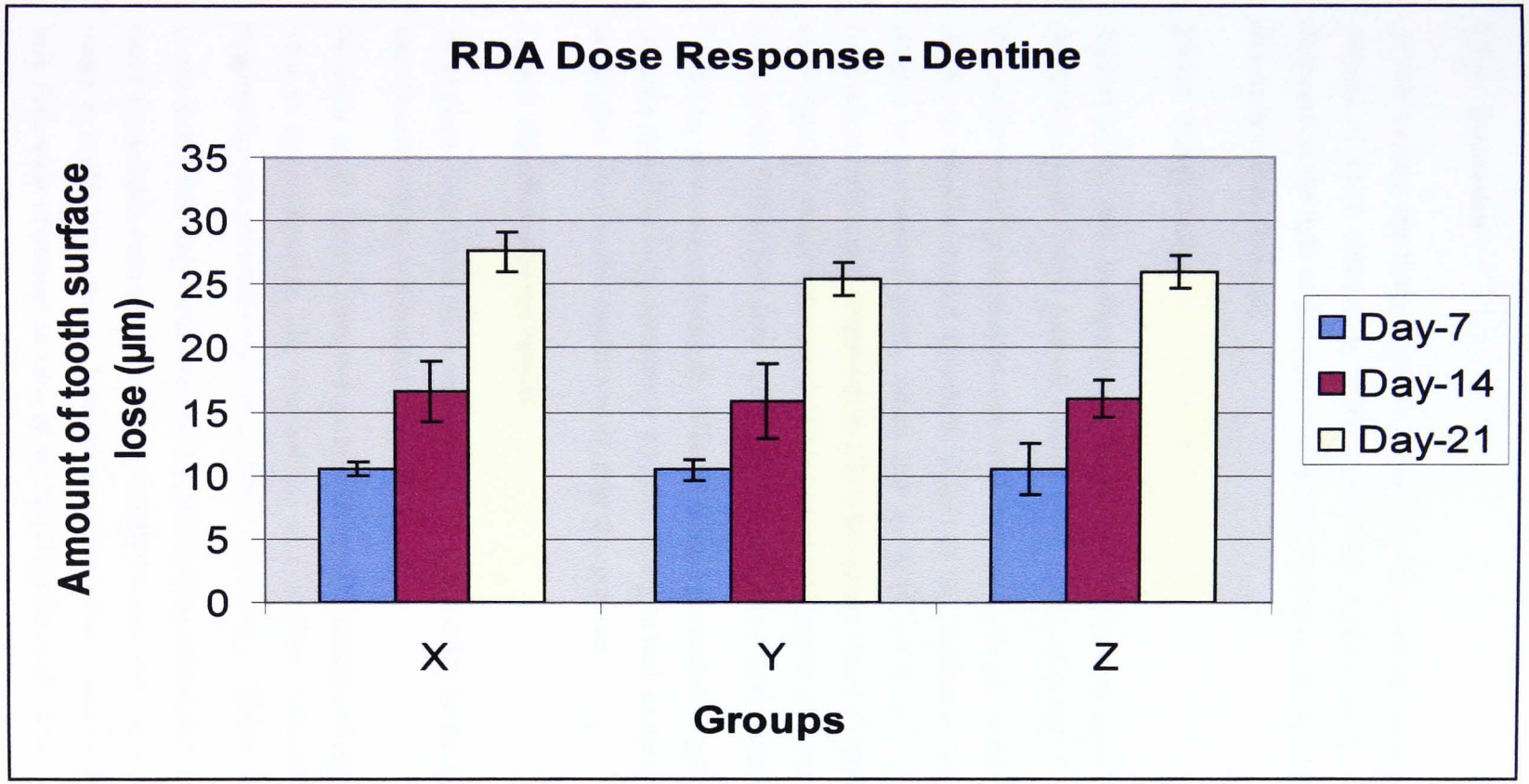
surface loss caused by using a 21-day pH cycling technique.

Days	Groups	Mean Diff (µm)	SD (µm)	Confidence Interval (95%) **		Sig.** (2-tailed)
				Min	max	
Day - 7	RDA 140 VS RDA 160	0.120	0.793	-0.543	0.783	0.681
	RDA 140 VS RDA 40	0.048	1.909	-1.549	1.644	0.946
	RDA 160 VS RDA 40	-0.073	2.131	-1.854	1.709	0.926
Day - 14	RDA 140 VS RDA 160	0.726	3.916	-2.548	3.999	0.616
	RDA 140 VS RDA 40	0.560	3.016	-1.962	3.081	0.616
	RDA 160 VS RDA 40	-0.166	2.958	-2.639	2.307	0.878
Day - 21	RDA 140 VS RDA 160	2.194	2.125	0.418	3.971	0.022*
	RDA 140 VS RDA 40	1.613	2.086	-0.131	3.356	0.065
	RDA 160 VS RDA 40	-0.582	1.427	-1.775	0.612	0.287

*** Significance**

**** Paired sample t-test was used for calculating the confidence intervals**

Figure 3.12: The effect of three different RDA content in toothpastes on the amount of enamel surface loss caused by using a 21-day pH cycling technique. (Toothpaste X: 140 RDA; Toothpaste Y: 160 RDA; and Toothpaste Z: 40).



3.5.4 Discussion

In this section, the findings of the effects of three matched toothpastes except for the amount of silica content on dental hard tissue erosion under erosive conditions is discussed in the light of the current literature. Moreover, the methodology employed in this study is also discussed.

3.5.4.1 Study Design

Toothbrushing with toothpaste was identified as the main agent in dentine abrasion (Bartlett & Smith, 2000; Addy & Hunter, 2003, Hunter *et al.*, 2002).

The major factor in enamel/dentine wear appears to be the relative dentine abrasivity (RDA) or relative enamel abrasivity (REA) of the toothpaste, which is its abrasivity relative to a standard paste, which has an RDA set at 100, determined using an International Standards Organisation (ISO) laboratory test. ISO stipulates that the RDA of toothpastes should not exceed 250 but most toothpastes in developed countries have RDA $\leq$ 100. Difficulties arise in extrapolating RDA to clinical outcomes.

Therefore, as we are studying the effect of using a preventative agent (toothpaste) which contains abrasives in its contents, it was interesting to look at the effect of abrasivity in our project. Our *in vitro* model was used for this purpose.

3.5.4.2 Discussion of the results

Toothpastes with lower RDA values showed no reduction in the amount tooth surface loss for both enamel and dentine using our model.

Philpotts *et al.*, (2005) observed a close linear relationship between RDA and dentine wear *in vitro*. However, this was not the case in three other *in situ* models as this relationship was not clear (Addy *et al.*, 2002; Hopper *et al.*, 2003; Pickles *et al.*, 2005).

It was found that only dentifrices with high relative enamel abrasivities (REA) (>200) cause appreciable rates of enamel wear, usually because they use non-hydrated alumina, which is harder than enamel. Dentifrices with relative enamel abrasivity <10 produce very little wear of enamel *in vitro* or in situ (Philpotts *et al.*, 2005; Hopper *et al.*, 2003; Pickles *et al.*, 2005; Lussi *et al.*, 2004b).

Magalhães *et al.*, (2008a) in their *in situ/ex vivo* study as described above found that when abrasivities (toothbrushing) combined with fluoride decreased the difference of erosive loss compared to using placebo and fluoride toothpaste on the erosion of bovine enamel.

All previous mentioned studies showed that very low RDA/REA values (<10) could show a decrease of the amount of tooth surface loss if compared a very high RDA/REA value (>200). This was confirmed with our findings in this study.

3.5.5 Conclusion

In this 21-day *in vitro*, toothpaste with lower RDA values showed no reduction in the amount of tooth surface loss for both enamel and dentine in our model. The null hypothesis was accepted in this model.

3.6 Study-8: The Effect of Different Fluoride Levels in Toothpastes on Tooth Surface Loss of Deciduous and Permanent Enamel

3.6.1 Introduction

Erosion was first introduced in 1993 in the UK children's dental survey. Nunn *et al.* (2003) in a review of children's dental surveys found an increase in the prevalence of erosion in children aged between 4-18 year olds. A trend towards a higher prevalence of erosion amongst 4-6 year olds was found. Dugmore and Rock (2003) in a cohort study examined 1308 children at the age of twelve years and again two years later. Deep enamel or dentine lesions were found in 4.9% of the children at baseline and 13.1% in two years time. New or more advanced lesions were observed in 27% of the children over the study period. Furthermore, 12% of the erosion-free children at baseline developed erosion during the two year period.

Erosive damage can cause discomfort and dentine hypersensitivity. In addition tooth surface loss during childhood may compromise the developing dentition and children may require repeated dental visits and expensive restorative work (Lussi *et al.*, 2006). Therefore measures to prevent and control the disease are essential.

Softening of the enamel surface is an early manifestation of the erosion process (Lussi *et al.*, 1995). In the initial stage of erosion where a scaffold of mineral crystals still remains, the lesion can be remineralised. When the surface is completely lost, the erosion process cannot be reversed (Amaechi and Higham 2005). Tooth brushing of the eroded softened enamel and dentine increases tooth wear (Davis and Winter, 1980).

Recently a low abrasion fluoride dentifrice containing potassium nitrate (Pronamel) has been introduced in the market and gives promising results for the protection against enamel surface loss (Rees *et al.*, 2007). Products containing CPP-ACP work as a supplemental source of calcium and phosphate ions in the oral environment. The amorphous calcium phosphate is biologically active, and is able to release calcium and phosphate ions to maintain the supersaturated state, thus enhancing the remineralisation process (Shen *et al.*, 2001; Walker *et al.*, 2006).

Deciduous teeth are smaller than permanent teeth, the enamel is thinner, and there are morphological differences compared to permanent teeth. Therefore, when we compare between permanent and deciduous teeth, the erosive process reaches the dentine earlier and leads to an advanced lesion after a shorter exposure to acids (Hunter *et al.*, 2000).

Amaechi *et al.* (1999a) examined the substance loss of deciduous and permanent teeth after immersion in orange juice. They found a 1.5 times greater progression of erosive lesions into the enamel of the primary dentition compared to that of the permanent dentition. In contrast to these findings, Hunter *et al.* (2000) measured only small differences in the susceptibility to erosion of deciduous and permanent teeth. Maupomé *et al.*, (1999) investigated the effect of a cola drink on deciduous and permanent enamel incorporating an early salivary pellicle. There was no statistical significant difference between primary and permanent teeth after exposure to acidic beverages. In another study, 60 primary and 60 permanent human teeth were immersed for three minutes in 12 different beverages and foodstuffs. Surface microhardness was measured before and after exposure. Initial (baseline) surface microhardness was lower for primary teeth than for permanent teeth. In both primary and permanent teeth, no statistically significant differences in the decrease of microhardness were found for the two enamel types. Overall decrease was 27±17 KMH (mean±SD) for primary and 26±16 KMH for permanent teeth. The same pattern was found when enamel was immersed for 6 min in different beverages and foodstuffs (Lussi *et al.*, 2000 & 2005). Lippert *et al.*, (2004) used nanoindentation combined with atomic force microscopy to investigate the erosive effect of four different drinks on enamel at early stages *in vitro*. In this short-term experiment, deciduous enamel was not found to be more susceptible to erosion than permanent enamel. In another study, a longer immersion time of up to 30 min and a more aggressive softening solution (2% citric acid, pH 2.1, 37°C) was tested. Microhardness measurements showed that enamel surface hardness decreased proportionately with increased time of immersion, in all tooth specimen groups. When permanent teeth were compared to deciduous teeth, the differences in microhardness were found to be statistically significant, with deciduous teeth being softer than the permanent teeth, both at baseline and after immersion in acid (Johannson *et al.*, 2001). These *in vitro* studies on erosion of deciduous teeth found the enamel of primary teeth to be softer than that of permanent teeth, the *in vitro* susceptibility of these teeth to

softening revealed conflicting results, and demonstrated either a higher susceptibility of deciduous or permanent enamel to erosion.

From the above studies, it seems that the increased susceptibility of deciduous enamel to erosion does not appear to occur in the initial phase, but rather over time and/or with increasing softening power of the acid. This is of importance to the clinician given the reduced dimension of the deciduous dentition and the continuously increasing intake of soft drinks by children. In addition, softer enamel such as the enamel of deciduous teeth is more prone to abrasion (Amaechi *et al.*, 1999a), which may explain the clinical picture often seen in children with significant tooth surface loss. The overlapping of erosion with attrition and/or abrasion is probably more pronounced in deciduous than in permanent teeth.

3.6.1.1 Aims

1. To compare the effect of toothpastes with different fluoride concentrations on tooth surface loss of permanent and deciduous enamel *in vitro*. The null hypothesis was there is no difference in tooth surface loss of either deciduous or permanent enamel with the increase of fluoride concentration in toothpaste.
2. To compare tooth surface susceptibility of permanent and primary dentition. The null hypothesis was that there is no difference of tooth surface susceptibility between deciduous and permanent enamel.

3.6.2 Materials and Methods

Human enamel slabs were used in this study. Slabs were obtained from intact permanent premolars, extracted for orthodontic reason, and from intact primary molars.

3.6.2.1 Enamel and Dentine Slab Preparation

This section was discussed in section 2.4.1 (page 25).

3.6.2.2 Microhardness

Slabs included in this study were pre-assessed for microhardness before including them in the study. This was described earlier (see section 3.3.2.4 on page 78). The micro hardness used

3.6.2.3 Storage of Enamel Slabs

Once the slabs had been prepared, they were kept moist in de-ionised distilled water in micro-centrifuge tubes and left at room temperature. This process was repeated after finishing the experiment.

3.6.2.4 Test Methods

3.6.2.4.1 Surface Profilometry (Scantron ProScan2000)

Baseline measurements of the surface profile of the slabs were assessed using a surface profilometer (Scantron ProScan 2000) to ensure that the average height to the average depth range was ± 1.0 μm. The technique was discussed in section 3.2.2.3 (page 67).

3.6.2.5 Test products

The following 6 groups were assigned for this study:

1. Experimental toothpaste formulations with 0, 500, 1000 and 1426 ppm NaF.
2. Marketed control toothpaste (MC) with 1426 ppm NaF and 5% $KN0_3$.
3. A regimen of the experimental toothpaste (1426 ppm NaF) with a once weekly two minute application of a marketed high fluoride gel (HFG) 12500 ppm NaF

Eight enamel (primary/permanent) slabs were used in each group; therefore there were 12 groups in total. A modified cycling technique for 21 days was used for this study.

3.6.2.6 Experimental Protocol/Regime

This section was explained earlier in a detailed manner. However, I have summarised again as I felt it is necessary to include it to remind the reader about the regime at this stage.

A special tray with eight holes that fitted the resin blocks was used to hold the blocks. Resin blocks were secured in position using adhesive wax. The slabs were immersed in a static condition for two minutes five times daily in 0.3% citric acid (pH 3.6) for a period of 21 days. Citric acid was prepared by adding three grams of mono-hydrate citric acid to 1 litre of de-ionised distilled water. The pH was 2.65±0.05 and then NaOH was added slowly and the pH monitored using a pH electrode (VWR international Orion, Orion research, UK) during the process until the pH reached 3.60 at room

temperature. Each group of slabs (eight slabs) was immersed at room temperature in fresh 200 ml aliquots of citric acid each time. On each occasion, before immersion in citric acid, the slabs were taken out of the artificial saliva and rinsed with de-ionised distilled water (pH 6.85±0.05). The slabs were also rinsed in de-ionised distilled water after treatment before they were returned to the artificial saliva which was changed daily. There was artificial saliva for remineralisation and was used during day time. For night time storage a different artificial saliva was used. The artificial saliva composition used in this study is explained in tables ∫ Table 3.5*Table 3.6.

Between immersions in citric acid the slabs were left immersed in artificial saliva for 60 minutes to enable remineralisation. The slabs were kept in an incubator at 37.0°C at all times except while they were being immersed in citric acid. At the end of the cycling period, the slabs were rinsed with de-ionised distilled water and air dried. The nail varnish was then removed using acetone and the enamel surface was cleaned with ethanol to ensure that all residues were removed. The slabs were then kept moist in de-ionised distilled water in micro-centrifuge tubes and left at room temperature.

The codes for the slabs were randomly changed after treatment to keep the study blind. The measurements were repeated five times to check the reproducibility of the methods and to determine the standard deviations when assessing the sensitivity of the methods for detecting changes caused by the erosive challenge.

3.6.2.7 Statistics

SPSS statistical software package for Windows, version 13.0 (SPSS Inc., 1989-2002; LEAD technologies Inc., 1991-2000) was used for data analysis, and measuring the "*p*" values. A significance level of $p \leq 0.05$ was accepted using student *t*-test.

The standard deviations of the treatment measurements and the confidence interval of the changes from baselines were used to check the sensitivity of the methods in detecting the changes caused by the erosive challenge.

3.6.3 Results

There were similar trends in the amount of tooth surface loss for both permanent and deciduous enamel.

3.6.3.1 Tooth surface loss of deciduous enamel

All groups showed an increased tooth surface loss (TSL) with time. There was no statistical difference between all groups at day-7. However, at day 14 the difference of TSL between the low fluoride concentration (0, and 500 ppm NaF) and the high fluoride concentration groups (1450 ppm NaF) was significant. At day-21, the significance level was more obvious as there was a significant difference in the amount of TSL between the 500 ppm NaF and 0 ppm NaF groups. This was similar when TSL in the 0 ppm NaF group was compared to the other groups with higher than 500 ppm NaF (Figure 3.13, Table 3.22, and Appendix 36).

Figure 3.13: Effect of longitudinal erosive challenge (21 days) on surface loss of deciduous enamel.

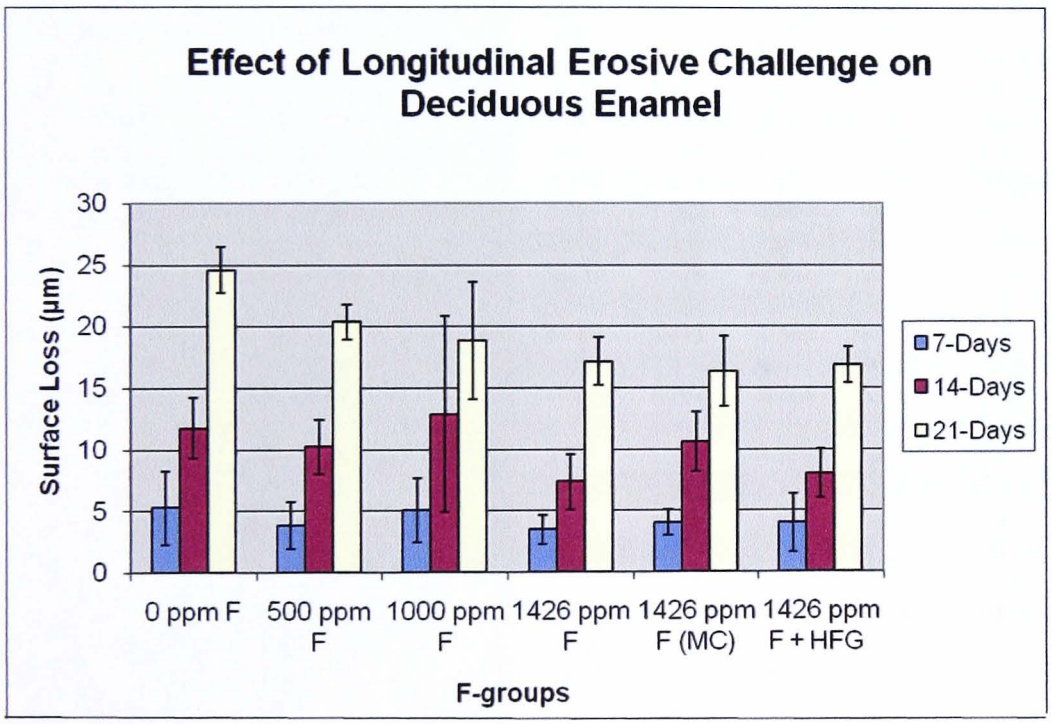


Table 3.22: Tooth surface loss of deciduous enamel after exposure for 21 erosive challenge using modified cycling technique and using six different fluoride treatment.

	F Group		Mean Diff	SE Diff	95% Confidence Interval		Sig. (2-tailed)**
					Lower	Upper	
Day-7	0 ppm	VS 500 ppm	1.404	1.248	-4.081	1.273	0.279
	0 ppm	VS 1000 ppm	0.189	1.393	-2.799	3.178	0.894
	0 ppm	VS 1426 ppm + HFG	1.276	1.355	-1.631	4.183	0.362
	0 ppm	VS 1426 ppm	1.231	1.118	-1.167	3.629	0.289
	0 ppm	VS 1426 ppm (MC)	1.792	1.131	-4.218	0.634	0.136
	500 ppm	VS 1000 ppm	-1.215	1.131	-3.640	1.210	0.301
	500 ppm	VS 1426 ppm + HFG	-0.128	1.084	-2.452	2.196	0.907
	500 ppm	VS 1426 ppm	0.387	0.785	-1.297	2.072	0.630
	500 ppm	VS 1426 ppm (MC)	-0.174	0.766	-1.817	1.470	0.824
	1000 ppm	VS 1426 ppm (MC)	1.602	1.000	-3.748	0.543	0.131
	1000 ppm	VS 1426 ppm + HFG	1.087	1.248	-1.590	3.764	0.399
	1000 ppm	VS 1426 ppm	1.042	0.985	-1.071	3.155	0.308
	1426ppm + HFG	VS 1426 ppm	-0.045	0.931	-2.041	1.951	0.962
	1426ppm + HFG	VS 1426 ppm (MC)	0.516	0.946	-2.546	1.514	0.594
	1426 ppm	VS 1426 ppm (MC)	0.561	0.556	-1.752	0.631	0.330
Day-14	0 ppm	VS 500 ppm	1.522	1.177	-4.047	1.002	0.217
	0 ppm	VS 1000 ppm	-1.110	2.959	-7.456	5.237	0.713
	0 ppm	VS 1426 ppm + HFG	3.779	1.124	1.367	6.190	0.005*
	0 ppm	VS 1426 ppm	1.144	1.220	-1.474	3.761	0.365
	0 ppm	VS 1426 ppm (MC)	4.416	1.186	-6.960	-1.873	0.002*
	500 ppm	VS 1000 ppm	-2.632	2.934	-8.924	3.661	0.385
	500 ppm	VS 1426 ppm + HFG	2.256	1.056	-0.009	4.522	0.051
	500 ppm	VS 1426 ppm	2.894	1.121	0.489	5.299	0.022*
	500 ppm	VS 1426 ppm (MC)	-0.379	1.158	-2.862	2.105	0.749
	1000 ppm	VS 1426 ppm (MC)	5.526	2.937	-11.826	0.774	0.081
	1000 ppm	VS 1426 ppm + HFG	4.888	2.913	-1.360	11.136	0.116
	1000 ppm	VS 1426 ppm	2.253	2.951	-4.077	8.583	0.458
	1426ppm + HFG	VS 1426 ppm	-2.635	1.104	-5.004	-0.266	0.032*
	1426ppm + HFG	VS 1426 ppm (MC)	0.638	1.066	-2.924	1.649	0.559
	1426 ppm	VS 1426 ppm (MC)	3.273	1.167	-5.775	-0.770	0.014*
Day-21	0 ppm	VS 500 ppm	4.203	0.823	-5.968	-2.437	0.000*
	0 ppm	VS 1000 ppm	5.731	1.809	1.850	9.611	0.007*
	0 ppm	VS 1426 ppm + HFG	7.727	0.829	5.948	9.505	0.000*
	0 ppm	VS 1426 ppm	8.270	1.195	5.707	10.833	0.000*
	0 ppm	VS 1426 ppm (MC)	7.458	0.946	-9.487	-5.429	0.000*
	500 ppm	VS 1000 ppm	1.528	1.762	-2.251	5.307	0.401
	500 ppm	VS 1426 ppm + HFG	3.524	0.721	1.978	5.070	0.000*
	500 ppm	VS 1426 ppm	3.255	0.853	1.426	5.084	0.002*
	500 ppm	VS 1426 ppm (MC)	4.067	1.122	1.660	6.474	0.003*
	1000 ppm	VS 1426 ppm (MC)	1.727	1.823	-5.637	2.182	0.359
	1000 ppm	VS 1426 ppm + HFG	1.996	1.765	-1.789	5.781	0.277
	1000 ppm	VS 1426 ppm	2.539	1.963	-1.672	6.750	0.217
	1426ppm + HFG	VS 1426 ppm	0.543	1.127	-1.874	2.960	0.637
	1426ppm + HFG	VS 1426 ppm (MC)	-0.269	0.859	-1.573	2.110	0.759
	1426 ppm	VS 1426 ppm (MC)	-0.812	1.216	-1.795	3.419	0.515

*** Statistical Significance**

**** Independent t-test used to measure "*p*" values**

3.6.3.2 Tooth surface loss of permanent enamel

The difference between groups in the amount of TSL was not consistent at days 7 and 14. However, this difference was closer in its trend at day 21 to the trend of TSL that occurred in deciduous enamel (Figure 3.14, Table 3.23, and Appendix 37).

Figure 3.14: Effect of longitudinal erosive challenge (21 days) on surface loss of permanent enamel.

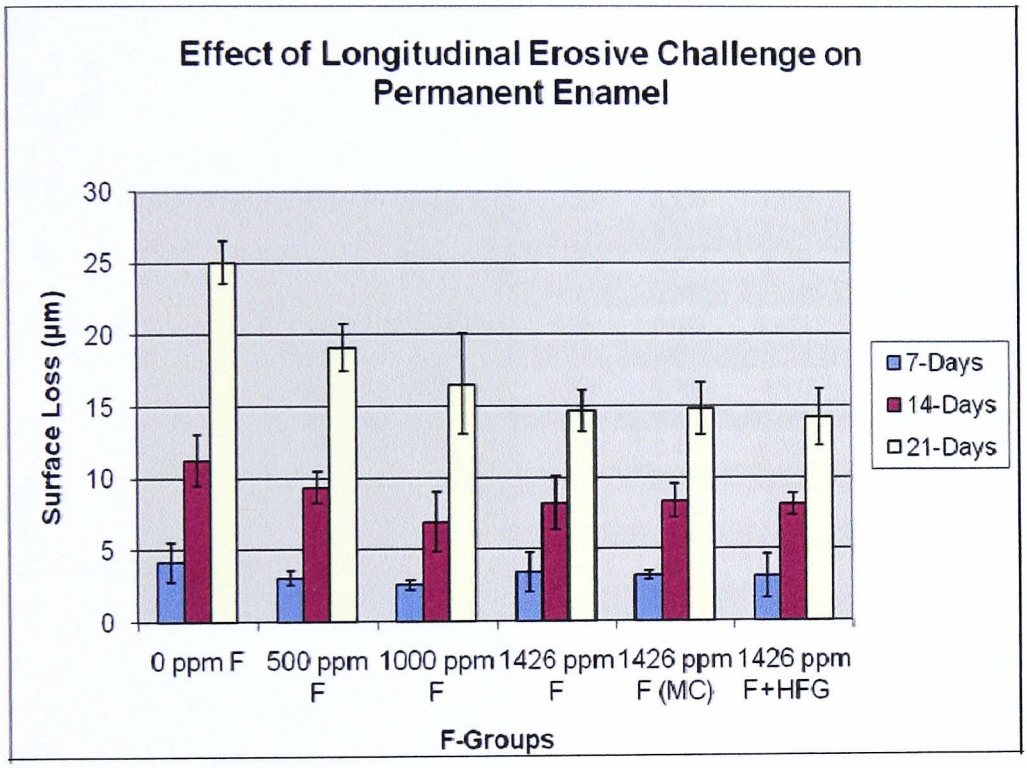


Table 3.23: Tooth surface loss of permanent enamel after exposure for 21 erosive challenge using modified cycling technique and using six different fluoride treatment.

	F Group		Mean Diff	SE Diff	95% Confidence Interval		Sig. (2-tailed)**
					Lower	Upper	
Day-7	0 ppm	VS 500 ppm	0.761	0.685	-2.230	0.709	0.286
	0 ppm	VS 1000 ppm	1.593	0.497	0.527	2.658	0.006
	0 ppm	VS 1426 ppm + HFG	1.052	0.728	-0.509	2.613	0.170
	0 ppm	VS 1426 ppm	0.960	0.493	-0.097	2.017	0.072
	0 ppm	VS 1426 ppm (MC)	0.761	0.685	-2.230	0.709	0.286
	500 ppm	VS 1000 ppm	0.500	0.210	0.050	0.949	0.032*
	500 ppm	VS 1426 ppm + HFG	-0.041	0.572	-1.267	1.185	0.944
	500 ppm	VS 1426 ppm	-0.332	0.516	-1.440	0.775	0.530
	500 ppm	VS 1426 ppm (MC)	-0.133	0.200	-0.563	0.297	0.518
	1000 ppm	VS 1426 ppm (MC)	-0.832	0.501	-0.242	1.906	0.119
	1000 ppm	VS 1426 ppm + HFG	-0.540	0.558	-1.736	0.656	0.349
	1000 ppm	VS 1426 ppm	-0.633	0.156	-0.967	-0.298	0.001*
	1426ppm + HFG	VS 1426 ppm	-0.092	0.554	-1.281	1.096	0.870
	1426ppm + HFG	VS 1426 ppm (MC)	-0.292	0.730	-1.275	1.858	0.696
	1426 ppm	VS 1426 ppm (MC)	-0.199	0.497	-0.867	1.265	0.694
Day-14	0 ppm	VS 500 ppm	1.914	0.735	-3.491	-0.336	0.021*
	0 ppm	VS 1000 ppm	4.343	0.970	2.263	6.423	0.001*
	0 ppm	VS 1426 ppm + HFG	3.136	0.683	1.671	4.601	0.000*
	0 ppm	VS 1426 ppm	2.887	0.747	1.284	4.490	0.002*
	0 ppm	VS 1426 ppm (MC)	3.026	0.908	-4.973	-1.078	0.005*
	500 ppm	VS 1000 ppm	2.430	0.837	0.635	4.224	0.012*
	500 ppm	VS 1426 ppm + HFG	1.222	0.476	0.202	2.242	0.022*
	500 ppm	VS 1426 ppm	1.112	0.764	-0.527	2.751	0.168
	500 ppm	VS 1426 ppm (MC)	0.974	0.564	-0.236	2.184	0.106
	1000 ppm	VS 1426 ppm (MC)	-1.318	0.992	-0.810	3.445	0.205
	1000 ppm	VS 1426 ppm + HFG	-1.207	0.791	-2.904	0.490	0.149
	1000 ppm	VS 1426 ppm	-1.456	0.847	-3.273	0.361	0.108
	1426ppm + HFG	VS 1426 ppm	-0.249	0.494	-1.308	0.811	0.623
	1426ppm + HFG	VS 1426 ppm (MC)	-0.110	0.714	-1.421	1.642	0.879
	1426 ppm	VS 1426 ppm (MC)	0.138	0.776	-1.802	1.526	0.861
Day-21	0 ppm	VS 500 ppm	-5.933	0.786	-7.619	-4.248	0.000*
	0 ppm	VS 1000 ppm	8.516	1.345	5.630	11.402	0.000*
	0 ppm	VS 1426 ppm + HFG	10.865	0.882	8.973	12.758	0.000*
	0 ppm	VS 1426 ppm	10.242	0.828	8.466	12.018	0.000*
	0 ppm	VS 1426 ppm (MC)	-10.370	0.732	-11.939	-8.801	0.000*
	500 ppm	VS 1000 ppm	2.583	1.369	-0.354	5.520	0.080
	500 ppm	VS 1426 ppm + HFG	4.932	0.918	2.962	6.902	0.000*
	500 ppm	VS 1426 ppm	4.437	0.775	2.775	6.098	0.000*
	500 ppm	VS 1426 ppm (MC)	4.309	0.866	2.450	6.167	0.000*
	1000 ppm	VS 1426 ppm (MC)	-1.854	1.339	-4.725	1.018	0.359
	1000 ppm	VS 1426 ppm + HFG	2.349	1.427	-0.711	5.410	0.277
	1000 ppm	VS 1426 ppm	1.726	1.394	-1.264	4.716	0.217
	1426ppm + HFG	VS 1426 ppm	-0.623	0.955	-2.671	1.424	0.637
	1426ppm + HFG	VS 1426 ppm (MC)	0.496	0.872	-1.376	2.367	0.759
	1426 ppm	VS 1426 ppm (MC)	-0.128	0.817	-1.881	1.625	0.515

*** Statistical Significance**

**** independent t-test used to measure "*p*" values**

3.6.3.3 Comparison between tooth surface loss of permanent and deciduous enamel

Deciduous enamel showed more TSL than permanent enamel at all time points. However, there was no statistical difference in the amount of tooth surface loss between deciduous and permanent enamel except at four occasions where permanent enamel showed significantly less TSL than deciduous enamel (Table 3.24).

Table 3.24: Comparison between tooth surface loss of permanent enamel and deciduous enamel after exposure for 21 erosive challenge using modified cycling technique and using six different fluoride treatment.

		Mean Diff	SE Diff	95% Confidence Interval		Sig. (2-tailed)**
				Lower	Upper	
Day-7	500 ppm (Dec) **VS** 500 ppm (Per)	0.782	0.691	-0.700	2.264	0.277
	1426 ppm (MC)(Dec) **VS** 1426 ppm (MC)(Per)	0.062	0.637	-1.304	1.429	0.923
	0 ppm (Dec) **VS** 0 ppm (Per)	1.093	1.159	-1.392	3.579	0.361
	1000 ppm (Dec) **VS** 1000 ppm (Per)	2.497	0.919	0.525	4.469	0.017*
	1426 ppm + HFG (Dec) **VS** 1426 ppm + HFG (Per)	0.870	1.012	-1.300	3.039	0.404
	1426 ppm (Dec) **VS** 1426 ppm (Per)	0.823	0.387	-0.007	1.652	0.052
Day-14	500 ppm (Dec) **VS** 500 ppm (Per)	0.849	0.877	-1.031	2.730	0.349
	1426 ppm (MC)(Dec) **VS** 1426 ppm (MC)(Per)	-0.933	1.036	-3.154	1.289	0.383
	0 ppm (Dec) **VS** 0 ppm (Per)	0.458	1.076	-1.850	2.766	0.677
	1000 ppm (Dec) **VS** 1000 ppm (Per)	5.911	2.922	-0.357	12.178	0.063
	1426 ppm + HFG (Dec) **VS** 1426 ppm + HFG (Per)	-0.185	0.757	-1.809	1.439	0.811
	1426 ppm (Dec) **VS** 1426 ppm (Per)	2.202	0.944	0.178	4.225	0.035*
Day-21	500 ppm (Dec) **VS** 500 ppm (Per)	1.232	0.772	-0.424	2.887	0.133
	1426 ppm (MC)(Dec) **VS** 1426 ppm (MC)(Per)	2.413	0.855	0.579	4.247	0.014*
	0 ppm (Dec) **VS** 0 ppm (Per)	-0.499	0.836	-2.292	1.294	0.560
	1000 ppm (Dec) **VS** 1000 ppm (Per)	2.286	2.094	-2.204	6.777	0.293
	1426 ppm + HFG (Dec) **VS** 1426 ppm + HFG (Per)	2.640	0.876	0.761	4.518	0.009*
	1426 ppm (Dec) **VS** 1426 ppm (Per)	1.473	1.189	-1.078	4.024	0.236

*** (Dec = Deciduous, Per = Permanent).**
**** independent t-test used to measure "*p*" values**

3.6.4 Discussion

3.6.4.1 Study Design

The six times daily dipping regime in an erosive challenge for five minutes on each occasion has been used (Amaechi *et al.*, 1998a and 1999c) previously in *in vitro* studies. However, this is probably an over-estimation of the "real life" situation. Therefore, we used a five times daily dipping regime under erosive challenge for two minutes on each occasion was used.

The dipping method was employed in the enamel studies instead of brushing. This was used to eliminate any abrasive component in this study. In addition, this was used to prevent extensive tooth surface loss.

A regimen of the experimental toothpaste (1426 ppm NaF) with a once weekly two minute application of a marketed high fluoride gel (HFG) 12500 ppm NaF was used in this study. This regimen was added as it is a recommended protocol for children with high susceptibility for erosion in some European countries.

3.6.4.2 Data Handling and Statistics

The present study was a pilot study to make an initial evaluation of the method, erosive challenges, and fluoride effect on dental erosion prior to carrying out a more extensive research project. In view of this, the results were interpreted in terms of confidence intervals and standard deviations.

3.6.4.3 Discussion of the results

The effect of fluoride in reducing tooth surface loss of enamel (permanent/bovine) is still controversial. Some studies showed that fluoride improved the resistance of enamel to erosion (Attin *et al.*, 2003; Lussi *et al.*, 1993). However, other researchers showed that fluoride had no effect in reducing dental erosion (Kelly and Smith, 1988; Lussi *et al.*, 2004b).

There are few studies that have investigated the effect of fluoride on tooth surface loss of the dental enamel of deciduous teeth. This study showed that deciduous enamel is susceptible to tooth surface loss and this susceptibility is greater than for the permanent dentition. However, this trend was not significant. This confirms the findings that other researchers previously reported (Amaechi *et al.*, 1999a; Hunter *et al.*, 2000; Johannson *et al.*, 2001). On the other hand, deciduous enamel was not found to be more susceptible to erosion than permanent enamel (Lippert *et al.*, 2004, Lussi *et al.*, 2000).

A protective effect of fluoride on tooth surface loss was observed for both deciduous and permanent enamel with increasing levels of fluoride in toothpastes. This confirmed the trend of a fluoride dose response seen in our previous work.

The regimen of the experimental toothpaste (1426 ppm NaF) with a once weekly two minute application of a marketed high fluoride gel (HFG) 12500 ppm NaF did show a superiority over the other toothpastes used. This might be due to the short period of using this toothpaste (3 applications only).

3.6.4.4 Conclusion

4. A similar fluoride dose response effect was seen with both deciduous and permanent enamel. This response was significant between all fluoride groups versus placebo group at day 21. The first null hypothesis in our aims was rejected in this model.
5. Deciduous enamel showed more surface loss than permanent enamel in this model. However, this was significant only in two comparison groups. The second null hypothesis in our aims was rejected. This is of importance clinically because of 'abuse' of soft drinks by the child population.

4 STUDY-9: THE EFFECT OF FLUORIDATED TOOTHPASTES ON SURFACE LOSS OF THE DENTAL HARD TISSUES UNDER EROSIVE CONDITIONS *IN SITU*

4.1 Introduction

Tooth wear is recognised as a major problem in both children and adults (Nunn *et al.*, 2003; Smith & Robb, 1996). It has a multi-factorial aetiology and is generally initiated following dissolution of mineralised tooth structure after contact with acids that are introduced into the oral cavity from intrinsic (e.g. gastroesophageal reflux, vomiting) or extrinsic sources (Smith & Robb, 1996). Prevalence data from cross-sectional UK studies indicates a progressive increase of dental erosion amongst young people with increasing age. This increase in erosive wear is evident in a greater overall number of teeth, on more surfaces with higher levels of bulk surface tissue loss. Dental erosion was associated significantly with increased soft drink consumption (Dugmore & Rock, 2004; Millward *et al.*, 1994; Milosovic *et al.*, 1997; Al-Dlaigan *et al.*, 2001).

Previous studies have explored ways to limit the erosive potential of foods and drinks (Attin *et al.*, 2003, Lussi *et al.*, 1993). The effect of the addition of mineral supplements to 1% citric acid on erosion of bovine enamel under controlled conditions has been investigated previously (Attin *et al.*, 2003). Addition of calcium, phosphate or fluoride to the citric acid solution resulted in significantly lower microhardness values (less length indentations) compared with the controls. An enhanced effect was seen when all three were added together. The formation of the CaF_2-like layer on the tooth surface would act as a 'barrier' against acid attacks. This layer provides some additional mineral to be dissolved during an acid attack before the underlying enamel is attacked (Ganss *et al.*, 2001). It is still controversial if these particles can be formed on sound tooth surfaces in vivo and in reasonable time. It has, however, been shown *in vitro* that KOH-soluble fluoride globules precipitate within a short time and in a higher amount when a low pH fluoride solution is used (Larsen & Richards, 2001; Petzold, 2001). The study by Larsen and Richards (2001) further showed a beneficial effect of saliva on the formation of

calcium fluoride-like material. Both a low pH of a fluoride solution with some subsequent loss of mineral and the calcium-rich saliva seem to be important factors in providing the system with calcium. It follows that deduction of a ranking for the *in vivo* erosivity of different acidic food and drinks based on pH, titratable acidity, Ca, P and F is rather complicated if not impossible. Besides these chemical factors, behavioural factors (such as eating and drinking habits, diets high in acidic fruits and vegetables, excessive consumption of acidic foods and drinks, oral hygiene practices) and biological factors (such as saliva flow rate, buffering capacity, acquired pellicle, dental anatomy and anatomy of oral soft tissues, physiological soft tissue movements) also have to be taken into account.

It has been found that an increased erosive capacity of different juices and foodstuffs was associated with a lower phosphate and fluoride content (Lussi *et al.*, 1993). Furthermore, the addition of fluoride (1 ppm) to erosive drinks significantly reduced their potential to cause erosion (Ganss *et al.*, 2008). The role of remineralising agents in preventing erosive damage has been the subject of increasing interest. In the same study it was shown that the application of fluoridated toothpaste significantly decreased the amount of enamel surface loss compared with no treatment control. Similarly the presence of fluoride as an ion in saliva has also been shown to have a protective effect on enamel under erosive conditions (Amaechi & Higham, 1998a).

It has also been reported recently that stannous fluoride (SnF_2) alone or mixed, in solution, with amine fluoride (AmF) had a significantly better protective effect on tooth surface loss of human enamel compared to sodium fluoride (NaF), AmF or NaF with AmF (all at 250 ppm F) (Ganss *et al.*, 2008). However all fluoride toothpastes had a beneficial effect in this model. More recently researchers have demonstrated effects for different fluoride sources and delivery formats using both *in vitro* and *in situ* investigations (Zero *et al.*, 2006; Ganss *et al.*, 2004b). Overall, formats containing fluoride such as toothpastes and mouthrinses have also been shown to provide varying degrees of protection.

The effect of xylitol, fluoride and a xylitol/fluoride combination has been studied on the erosion of bovine enamel by pure orange juice *in vitro*. There was a significant beneficial effect in reducing mineral loss when xylitol and fluoride were used in combination (Amaechi *et al.*, 1998a).

Mechanical factors, such as abrasion from toothbrushing or toothpaste attrition acting on the demineralised surface, can lead to tissue loss (Davis and Winter,1980; Hooper *et al.*, 2003; Amaechi *et al.*, 2003). In 1980, Davis and Winter found that following an erosive challenge (grapefruit) the demineralised enamel tissues were removed after brushing. The abrasivity of two different toothpastes were more than the control group but the difference was not significant (Hooper *et al.*, 2003). Amaechi *et al.*, (2003) suggested that dental erosion is caused by an erosive agent and subsequent abrasion by the action of the surrounding oral soft tissues and through food mastication. In addition, the abrasive effect of the oral soft tissues may contribute to the site-specificity of dental erosion

Our preliminary work (section 3.4) showed that dental erosion of enamel was reduced when using fluoride toothpaste (1100 ppm F and 1450 ppm F). The amount of surface loss increased over the period of treatment (21 days). Therefore, we aimed to use the cycling period to 21 days in this study, to include enamel and dentine samples and to investigate the effect of tooth brush abrasion on tooth surface loss.

Brushing with fluoridated toothpastes represents one of the most efficient ways of delivering remineralising agents on a daily basis, as part of regular oral hygiene procedures.

In summary, there is little data in the literature on the relative protection provided by various concentrations of fluoride in toothpastes against a prolonged erosive challenge.

4.1.1 Beneficial application of fluoride by toothpastes prior to an erosive attack

Davis and Winter in 1977 studied the effects of application of a fluoride toothpaste on enamel, prior to it being exposed to some erosive agents (orange juice, potassium acetate, neutral EDTA). A statistically significant difference was observed between the control and test groups, and therefore, they recommended brushing for one minute with (0.8%) sodium monofluorophosphate/calcium carbonate toothpaste before eating a low pH meal in order to decrease the erosive losses of enamel.

Similar results were observed by other *in vitro* and in situ studies (Munoz *et al.*, 1999, Ganss *et al.*, 2001; Ganss *et al.*, 2004b; Hughes *et al.*, 2004), in which a beneficial effect of fluoride treatment with toothpaste before the erosive incidence was detected.

4.1.2 In Situ models

In situ models involve the use of appliances or other devices which create defined conditions in the oral environment in order to simulate natural processes (Zero, 1995). In the first experimental intra-oral models small gold cups (Bunting *et al.*, 1926) or gold plates (Nygaard Østby *et al.*, 1957) were used when studying demineralisation in vital teeth. In 1964, Koulourides and Volker introduced an in situ model (Intra-oral Cariogenicity/ICT model) to study the cariogenicity of various types of foods and suggested this method as suitable to determine the ability of topically applied substances to limit tooth decay. Since then, in situ models, modified from Koulourides' first model, have been widely used in various aspects of dental research serving as an intermediate step between test procedures in animals and *in vitro* investigations on the one hand and clinical or field trials on the other (Manning and Edgar, 1992; Clasen and Øgaard, 1999).

Therefore in situ models have been widely used for the study of remineralisation and demineralisation of enamel and dentine, the effect of fluoride and of various chemotherapeutic agents on dental caries, the cariogenicity of foods, the effect of erosive and abrasive agents on enamel and dentine and the prevention of non-cariogenic tooth surface loss.

The major advantage of this type of study is that experiments are conducted in the human oral environment as opposed to the extra-oral laboratory conditions of an *in vitro* study or to animal studies, which are questionably related to the human oral environment. However, in contrast with the *in vivo* situation, where various factors such as dietary eating habits, plaque of varying composition and thickness, and a pellicle coated tooth surface (Bowen, 1983; Kleinberg *et al.*, 1983) introduce uncontrollable variables in the experiment, in situ models standardise many of these variables. In addition, these models allow for the application of various basic scientific analytical techniques, resulting in a more sensitive and scientifically valid methodology, compared to the in vivo experiments where the cruel clinical and radiographic tools of caries diagnosis are the only option. Finally the short duration of these studies solves many of the ethical problems that the clinical trials face, and they are not as costly. Despite all these advantages, in situ studies have some disadvantages compared to clinical trials. Due to the small number of subjects that in situ

studies involve (up to 40) there is a debate as to whether the results can be applied to the general population. On the other hand, these studies are highly demanding, as far as laboratory and clinical knowledge and skills are concerned, and they are dependent on the subject's compliance.

4.1.3 Saliva

Saliva has been defined as consisting of fluids derived from the major salivary glands (the parotids, submandibular and sublingual), from the minor glands of the oral mucosa and traces from the gingival exudates (Newbrun, 1989). A very thin film of saliva of approximately 1-10 μm is present over the surfaces of the oral cavity with never usually more than 0.5ml present in the mouth at once (Newbrun, 1989). Saliva provides protection against the development of dental caries or erosion. Finn and Klapper (1954) found that desalivated hamsters developed many more carious teeth than those with intact salivary glands.

The composition of the various components in saliva varies with the degree of stimulation and the nature of stimulation (Newbrun, 1989). For example, stimulation of parasympathetic receptors leads to secretion of water and electrolytes whereas stimulation of sympathetic receptors leads to increased protein secretion (Edgar and O'Mullane, 1990).

4.1.3.1 Unstimulated salivary flow

The average unstimulated salivary flow rate is approximately 0.3 ml per minute. However, several factors affect this salivary flow rate. Dawes (1972) found that unstimulated whole saliva showed significant circadian rhythms for flow rate and for concentrations of sodium and chloride but not for protein, potassium, calcium, phosphate or urea. Stimulated parotid saliva showed significant circadian rhythms for the concentrations of protein, sodium, potassium, calcium and chloride but not for phosphate or urea. It was shown that the salivary flow rate peaked during the afternoon but almost dropped to zero during sleep. Twenty percent of unstimulated saliva was produced by the parotid glands, 65% by the submandibular glands, 7-8% by the sublingual glands and 7-8% by the minor mucous glands (Edgar and O'Mullane, 1990).

4.1.3.2 Stimulated salivary flow

Several factors, including psychological factors, may influence the stimulated salivary flow rate, the average of which is approximately 1.5 ml per minute. Acid is the most powerful of the four gustatory stimuli with salt, bitter and sweet stimulating saliva flow to a lesser extent (Edgar and O'Mullane, 1990). Mechanical stimulation causes a much smaller increase in salivary flow rate compared with acid stimulation (Watanabe and Dawes, 1986). However, few studies have considered the effect that food might have on salivary flow rate. Dawes (1983) found that the most important parameters affecting sugar clearance from the oral cavity were the unstimulated salivary flow rate, the maximum volume of saliva in the mouth before swallowing and the residual volume in the mouth immediately after swallowing. Lienthal (1955) suggested that rapid salivary flow and low viscosity tended to be associated with increased sugar clearance. This issue of retention of carbohydrate in the oral cavity has major implications for the cariogenic potential of food.

4.1.4 Parameters of the in situ models

The most important experimental parameters that can be controlled by the investigator and that influence the response of in situ models are (Curzon and Hefferren, 2001):

1. The physical design of the model
2. The characteristics of the subject panel
3. The type of the hard tissue substrates
4. The method of assessing mineral status
5. The study design

4.1.4.1 The physical design of the model

The intra-oral models developed in dental research are of different designs such as the classical partial denture model described by Koulourides *et al.* (1974), or the orthodontic banding model developed by Øgaard and Rølla (1992). The type of the design has a major impact on the response and reproducibility of the model, as "each in situ model develops a unique set of environmental conditions for plaque growth, dietary carbohydrate substrate diffusion, retention in the mouth, and salivary access" (Wefel, 1995). It is therefore

essential to choose the physical design of the model according to the purpose or objectives of the study.

4.1.5 Removable appliances

In this in situ model design the dental slabs are mounted bilaterally on a palatal removable appliance.

4.1.5.1 Palatal appliance

Brudevold *et al.* (1984) and Zero *et al.* (1992) used a palatal acrylic plate carrying up to eight enamel blocks, which were covered with a layer of extra-orally cultivated bacteria. Changes in these surfaces were observed over short time periods (few hours). However, the palatal position of the specimens confined the clinical relevance of the results, whereas it was questionable whether this model was suitable for experiments of long duration (ten Cate, 1992) as the S. mutans cells covering the enamel blocks were eliminated from the mouth within one to two weeks (Brudevold *et al.*, 1984).

4.1.5.2 Banding models

Nygaard Østby *et al.* (1957) and later Øgaard *et al.* (1986) used an *in situ* model where retention sites were created by the placement of bands around teeth that were scheduled to be extracted. The microflora behind the bands was similar to that associated with natural caries (Ameberg *et al.*, 1976). This model was the closest to natural processes from all of the *in situ* models and therefore could actually be considered an *in vivo* model. It has been more frequently used in demineralisation studies but it can be used as a remineralisation model as well (Øgaard *et al.*, 1988). However, the research had to overcome many shortcomings when conducting this type of study. Firstly, although the subjects were children, the number of teeth which were suitable for these studies was relatively small and no baseline measurements could be obtained. Secondly, it was impossible to use the same panellists in subsequent studies (ten Cate, 1992). Finally, there may have been variations in lesion development even within the dentition of the same subject (Clasen and Øgaard, 1999).

4.1.5.3 The "attached specimen" model

This model, introduced in 1992 by Manning and Edgar, used orthodontic brackets or a pre-formed resin holding a small specimen, which was cemented to the buccal surface of a tooth. Although, compliance did not pose a problem with this model, the subject's discomfort and the cleansing effect of the mucosa could be considered as disadvantages (ten Cate, 1992).

4.1.5.4 The partial-denture model

This used the space available in the dental arch to place specimens in a partial prosthetic appliance (Koulourides and Volker, 1964; Dijkman *et al.*, 1986; ten Cate and Rempt, 1986) that varied from a single crown to almost an entire quadrant, or it made use of either slots created in cemented crowns or of full dentures. The specimens could be positioned on the buccal, palatal or interproximal surfaces.

4.1.5.5 Single-section models

Creanor *et al.* (1986) introduced a new de- and remineralisation model, where instead of enamel slabs, single sections of approximately 100-120 μm thickness were mounted in a trough of a lower removable appliance. Other researchers (Wefel *et al.*, 1987; Strang *et al.*, 1987) used the same model, advocating that the use of single sections provided the advantage of sequential measurements throughout the study; this rendered the model more sensitive than those which used enamel blocks, as the quantitative microradiography or the polarised light microscopy technique could be used for sequential measurements only for the single-section model (Wefel *et al.*, 1987). In addition, with this model sections could easily be placed in approximal sites that allowed for these caries-prone areas of the dentition to be mimicked (Creanor *et al.*, 1986). On the other hand, these mounting sites protected the specimens from damage as compared to the "more exposed" buccal surfaces (Wefel *et al.*, 1987). Furthermore, single sections were more uniform than enamel blocks excluding, therefore, biological variation during the longitudinal investigation (ten Cate and Exterkate, 1986). However, it has been shown that even after using single sections

subjected to a standardised, pre-intra-oral caries challenge, the enamel variance was unavoidable (Mellberg *et al.*, 1988).

ten Cate and Exterkate (1986) compared the demineralisation of enamel in sections with that in bulk specimens and found that 25% more mineral was removed from the sections after exposing both to the same acid attack. However, Strang *et al.*, in 1988 who repeated the same experiment but only changed the preparation of the single sections reported no significant difference between the rate of demineralisation between enamel blocks and single sections.

A variation of this *in situ* model was the thin-section sandwich model introduced by Mellberg *et al.* (1986) where several layers of enamel were sandwiched between protective sheets of plastic.

4.1.6 The type of hard tissue substrate

For *in situ* studies various types of dental tissues have been used. These included primarily human (mainly permanent rather than primary teeth) and bovine enamel, and human dentine. The specimens might have been in a natural, ground, or decalcified state. Bovine enamel gave reproducible measurements, especially when the outer surface of approximately 100-200 μm was removed. The chemical composition of bovine enamel varied less than that of human enamel and had a lower fluoride concentration (Øgaard and Rølla, 1992, Clasen and Øgaard, 1999). Lesion development in bovine enamel was faster than in human due to the lower porosity of the latter but the caries response was qualitatively similar for both types (Mellberg, 1992).

As far as the status of the hard dental tissues was concerned, a natural enamel surface was preferable than an abraded one, as the outer surface was richer in fluoride. However, many evaluative techniques required a flat surface for reproducible measurements. Finally, in remineralisation studies where pre-formed lesions were created on the specimens, the method used played an important role on the response, the reliability and validity of the *in situ* model (Zero, 1995).

4.1.7 The characteristics of the subject panel

Demographic characteristics of the panellist such as age, gender and racial/ethnic background, the medical and dental health status, their background fluoride exposure, behavioural factors, dietary factors and salivary factors should be taken into consideration prior to the subjects' selection (ten Cate, 1992; ten Cate *et al.*, 1992; Zero, 1995).

4.1.8 The study design

The study design variables, as described by Zero in 1995, included the number of subjects, the use of appropriate controls, the type of study design (cross-over versus monadic), the length of the test periods, the dietary challenge, the method and frequency of delivery of the test agent, the use of standardised lead-in procedures, the length of the wash-out period, the use of compliance indicators, and the experimental parameters measured.

4.1.9 Rationale for Study Design

This model was used previously for some *in vitro* studies in our laboratory. It showed the ability to produce comparable results between fluoridated toothpastes. Following the results of the earlier *in vitro* studies using this model, it seemed appropriate at this stage to investigate this model using in situ appliances. The study design in this protocol used methodology which had been previously validated and accepted by the regulatory authorities (Ethical Committee in Leeds).

Five daily two minute dippings in an erosive product and the twice daily use of toothpaste was planned for this study. This pH cycling regime was developed during our previous *in vitro* work and was shown to be sufficient for creating erosive lesions.

Our previous work also showed that comparable results could be detected after at least 16 days of cycling using this model, and therefore, the length of each study arm was set at 21 days.

Volunteers had to wear removable appliances which were placed on the palate. This was to comply with the standard position of similar devices that were used in previous *in situ* studies (West *et al.*, 1998; Hara *et al.*, 2009; Magalhães *et al.*, 2008b).

In this study, the effect of the erosive agents and the effect of toothpastes only were compared. The effect of abrasion caused by other sources was excluded to simplify the study as this was an exploratory study.

As mentioned previously, surface profilometry was used to measure the amount of surface loss and then to compare between groups. Surface profilometry was tested in our previous work and this method was shown to be sufficiently sensitive to detect differences.

The effect of sodium fluoride was investigated in this study. This was our main interest since this was the case with many researchers recently.

Hara *et al.* (2009) compared dentifrices containing similar sources/concentrations of fluoride on the remineralisation of eroded enamel *in situ*. They recruited fifty-three subjects in a double-blind crossover study with three randomly assigned dentifrice treatments: placebo (0 ppm NaF, PD); reference (1,450 ppm NaF, RD) and test (1,450 ppm NaF + 5% KNO_3, TD). They checked the fluoride availability for each dentifrice before test (1-min fluoride release rate and enamel fluoride uptake). They used a total of 1,392 bovine enamel slabs and divided them randomly into the three balanced experimental groups. Each group had 464 bovine specimens in eight replicas within 58 experimental units. Each slab was individually immersed *in vitro* in 40 ml of fresh grapefruit for 25 minutes. Then, each subject wore a palatal appliance with mounted bovine enamel slabs (n=8) that was previously eroded. The subjects were instructed to wear the appliance for five mins then each subject brushed the buccal surfaces of their teeth using a toothbrush loaded with the assigned dentifrice for 25 seconds, creating a dentifrice/saliva slurry. The toothbrush did not come into contact with the specimens during brushing. The slurry was then swished around the appliance for one minute in order to promote contact with the experimental surfaces of the specimens. The appliance was worn for the following four hours and then the specimens were collected. The same procedure was repeated for the subsequent phases but changing the dentifrice provided to the subject, according to the crossover experimental design. A second erosive challenge was applied after the *in situ* scenario with a similar erosive challenge to the first erosive challenge. Surface microhardness was determined before and after the *in vitro* erosive challenge, after in situ remineralisation and after a second *in vitro* erosive challenge. ANOVA and pairwise comparisons were performed (α =

0.05). TD was found to be superior to RD in the fluoride release tests, but similar to RD in the enamel fluoride uptake test. The mean percent surface microhardness recovery was 21.9 (standard deviation 8.0) for PD, 28.6 (8.0) for RD and 36.0 (8.0) for TD. The mean percent relative erosion resistance change was -58.8 (12.7) for PD, -31.3 (12.7) for RD and -27.3 (12.6) for TD. Both fluoride-containing dentifrices provided superior remineralisation ($p \leq 0.001$) and erosion resistance ($p \leq 0.001$) compared to PD. The percent surface microhardness recovery demonstrated by the TD was significantly greater than for the RD ($p \leq 0.001$). There was no significant difference ($p=0.073$) between TD and RD in relative resistance to further erosive challenge. The researchers suggested that fluoride dentifrices provided erosion resistance for bovine enamel. This study compared the effect of fluoride toothpaste on the surface loss of bovine enamel using a short exposure to an erosive challenge and a single application of fluoride with a short *in situ* scenario. On the other hand, Magalhães *et al.* (2008b) in an in situ/ex vivo study, assessed the effect of different concentrations of fluoride in dentifrices on dentine subjected to erosion or to erosion plus abrasion. Ten volunteers took part in the crossover and double-blind study which was performed in three phases (seven days). Subjects wore acrylic palatal appliances containing four bovine dentine blocks divided into two rows: erosion and erosion plus abrasion. The blocks were subjected to erosion by immersion *ex vivo* in a cola drink (60 s, pH 2.6) four times daily. During this step, the volunteers brushed their teeth with one of three dentifrices D (5,000 ppm F, silica); C (1,100 ppm F, silica) and placebo (22 ppm F, silica). Then, the respective dentifrice slurry (1:3) was dripped onto the dentine surfaces. While no further treatment was performed in one row, the other row was brushed using an electric toothbrush for 30 s ex vivo. The appliances were replaced in the mouth and the volunteers rinsed with water. Dentine loss was determined by profilometry and analyzed using two-way ANOVA/Bonferroni test (a=0.05). Dentine loss after erosive-abrasive wear was significantly greater than after erosion alone. Wear was significantly higher for the placebo (3.58±0.71 µm) than for the D (2.58±0.80 µm) and C (2.45±0.5 µm) dentifrices. Fluoride toothpastes were not significantly different from each other. The investigators concluded that the presence of fluoride concentrations around 1,100 ppm NaF in dentifrices was important to reduce dentine wear by erosion and erosion + abrasion, but the protective effect did not increase with fluoride concentration. As in the study of Hara *et al.* (2009),

this model used a different protocol than our model. However, the results indicated a similar scenario.

Ganss *et al.* (2007a) studied the stability of CaF_2-like precipitates on enamel and dentine under neutral or acidic conditions and compared *in vitro* and in situ results. They used human enamel and dentine specimens. Slabs were treated with fluoride (Elmex fluid, five minutes) and subjected to erosive demineralisation (Sprite light: 3/day each; 30 seconds *in vitro*, two mins in situ) or stored under neutral conditions for four days *in vitro* or seven days in situ. KOH-soluble fluoride was determined using an ion-selective electrode. Between the acid attacks, specimens were stored in a remineralisation solution (*in vitro*) or retained in the oral cavity (four volunteers for enamel and dentine each). They found that high amounts of KOH-soluble fluoride were gained (between 77.9±12.3 and 96.0±46.4 $\mu g/cm^2$). Under neutral conditions *in vitro*, a significant decrease on enamel (16.2±5.0 $\mu g/cm^2$) and dentine (18.6±10.5 $\mu g/cm^2$) was found, which was more severe under acidic conditions (6.3±3.0 and 5.1±2.1 $\mu g/cm^2$, respectively). However, they found that under *in situ* conditions, KOH-soluble fluoride was stable on enamel under neutral (42.3±12.6 $\mu g/cm^2$) as well as under acidic conditions (54.1±17.4 $\mu g/cm^2$). For dentine, the dissolution kinetics of KOH-soluble fluoride was similar to the *in vitro* conditions, but the loss was less severe (45.3±12.9 $\mu g/cm^2$) under neutral and 8.8±6.4 $\mu g/cm^2$) under acidic conditions. *In vitro*, more KOH-soluble fluoride was lost under erosive compared to neutral conditions. The intra-oral environment was considerably protective for CaF_2-like precipitates especially on enamel. Hunter *et al.*, (2003) investigated *in vitro* the effect of different fluoride preparations on erosion attributed to citric acid and citric acid-based soft drinks. Flat enamel specimens embedded in epoxy resin were used. Slabs were taped except a 2 mm window of exposed enamel. Groups of specimens were exposed to citric acid and soft drinks with and without the addition of sodium fluoride or exposed to the same solutions after pre-treatment with fluoride products. Enamel loss was measured by profilometery after 10, 20 and 30 min of acid exposure. The different acidic solutions varied significantly in the amount of erosion produced both with and without the addition of fluoride. In addition, the different fluoride products differed significantly in the protective effect afforded. Both fluoride application methods reduced in mean terms, enamel erosion at all time points and by all acidic solutions. The majority of differences

were <25% and as the study was powered to show differences as significant at or above this level few reached statistical significance. Fluoride applied to enamel either in acidic solutions or as a pre-treatment reduced enamel erosion; however, the actual clinical benefit appeared to be low.

4.1.10 Demineralisation and remineralisation evaluation techniques

Evaluation techniques for *in situ* models were used to quantify the mineral that had been lost or gained and to identify the position, with respect to the outer surface, that the demineralisation or remineralisation had occurred (Arends and ten Bosch, 1992). The methods used for this assessment varied in their degree of sophistication and quantitative capabilities, that ranged from indirect measures of mineral loss or gain (e.g. surface microhardness) to direct measures (e.g. microradiography) (White *et al.*, 1992).

The evaluation method selected depended on the study design (e.g. need for single measurement or for repeated measurements), the study model (e.g. use of natural teeth or intra-oral devices) and the methods specification (e.g. sensitivity, reproducibility). In addition, the available resources such as the available expertise, the availability of the equipment, the cost of purchase or construction, time restrictions and cost of measurements, played an important role in the selection of the appropriate evaluation technique (ten Bosch and Angmar-Mansson, 1991). Several quantitative techniques have been developed for this purpose:

1. Wet chemical analysis
2. Transverse microradiography and longitudinal microradiography
3. Microhardness testing (surface and cross-sectional)
4. Polarised light microscopy
5. Iodide permeability test
6. Iodine absorptiometry
7. Light scattering
8. Scanning electron microscopy
9. Light microscopy
10. Confocal laser scanning microscopy
11. Laser-induced fluorescence methods

12. Ultrasound microscopy

13. Quantitative X-ray microtomography (Wong *et al.*, 2004)

4.1.11 Quantitative techniques for studies of erosion/abrasion

Various techniques have been used for the laboratory assessment of enamel surface loss:

1. Surface hardness and nano-indentation techniques
2. Profilometry
3. Microradiography
4. Chemical analysis
5. Microscopy techniques (SEM, ESEM)
6. Confocal laser scanning microscopy (CLSM)
7. Atomic force microscopy (AFM)
8. Secondary ion mass spectroscopy (SIMS)
9. Quantitative light-induced fluorescence (Barbour and Rees, 2004)

4.1.12 Good Clinical Practice (Note for Guidance on Good Clinical Practice, 1996).

Good Clinical Practice (GCP) is an international ethical and scientific quality standard for the design, conduct, performance, monitoring, auditing, recording, analysis, and reporting of clinical trials that ensures the data and the results reported are accurate and credible and that the trial subjects' rights, integrity and confidentiality are protected.

Guidelines to provide a standard for clinical trials were compiled first after the Second World War in the Nuremberg code, which set forth ten conditions that must be met to justify research involving human subjects. The two most important conditions were the need for voluntary informed consent of subjects and a scientifically valid research design that could produce fruitful results for the good of society. In 1964, the 18th World Medical Assembly developed the Declaration of Helsinki as a statement of ethical principles to provide guidance to physicians and other participants in medical research involving human subjects. Since then, the Declaration of Helsinki guidelines have been modified by later World Medical Assemblies.

In May 1996, in an attempt by the European Union (EU), Japan and the United States to comply to a unified standard and therefore for their regulatory authorities to mutually accept clinical data, the International Conference of Harmonisation (ICH) modified the guideline of Good Clinical Practice, which originated from the Declaration of Helsinki (ICH GCP). The guideline was developed with consideration of the current good clinical practices of the European Union, Japan, and the United States, as well as those of Australia, Canada, the Nordic countries and the World Health Organisation (WHO).

Every clinical trial should conform to this guideline in order to be accepted by the regulatory authorities. Additionally, any other clinical investigation that may affect the safety and well-being of human subjects should be conducted according to the principles established in this guideline.

4.1.12.1 The Principles of ICH GCP

1. Clinical trials should be conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinski, and that are consistent with GCP and the applicable regulatory requirement(s).
2. Any risk or inconvenience that could be predicted should be weighed against the anticipated benefit for the individual trial subject and society, prior to the initiation of the trial. Only if the risks are justifiable by the anticipated benefits should the trial be initiated and continued.
3. The greatest concern of the researcher should be to protect the rights, safety and well-being of the trial subjects and this should not be compromised for the sake of science and society.
4. Any investigational product should be adequately supported by clinical and non-clinical information in order for its use in the clinical trial to be justified.
5. Clinical trials should be scientifically sound, and described in a clear, detailed protocol.
6. The protocol should be reviewed and approved by an institutional review board (IRB)/independent ethics committee (IEC) prior to the initiation of the trial. The trial then should be conducted in compliance with this protocol.
7. A qualified physician or, when appropriate a qualified dentist should be responsible for the subjects medical care and for any medical decisions that are made on behalf of them.

8. Each trial subject should be sufficiently informed and trained prior to the trial.

9. Each trial subject should freely give consent prior to clinical trial participation.

10. All the records of the clinical trial should be stored appropriately in order to be accurately reported, interpreted and verified.

11. The subject's confidentiality should be protected according to the applicable regulatory requirement(s).

12. Investigational products should be manufactured, handled, and stored in accordance with applicable good manufacturing practice (GMP). They should be used in accordance with the approved protocol.

13. In order to assure the quality of every aspect of the trial appropriate systems with certain procedures should be implemented.

4.1.13 Consolidated Standards of Reporting Trials (CONSORT)

The broad goals of any reporting guidance are to improve the transparency and reporting of the specific design. Unfortunately, few reporting guides include an assessment of whether the reporting guidance achieves its intended objective, namely, improving the quality of reporting.

The Consolidated Standards of Reporting Trials (CONSORT) is an attempt to improve the reporting of randomised clinical trials. It was developed by a broad-based group of journal editors, biostatisticians and researchers, intimately involved in clinical trials, and was first published in 1996 (Begg *et al.*, 1996).

An early evaluation of the 1996 CONSORT Statement was published in JAMA (Moher *et al.*, 2001). Here, the authors conducted a comparative before-after evaluation. Pre-CONSORT (1994) and early post-CONSORT (1998) reports (n=148) of RCTs published in three CONSORT-adopting journals (BMJ, JAMA and The Lancet) were compared to 63 reports of RCTs published in one non-CONSORT-adopting journal (New England Journal of Medicine) during both time periods. Compared to 1994 reports of RCTs published in 1998, use of the CONSORT Statement was associated with improvements in the quality of reports of RCT.

Figure 4.1 and Table 4.1 show examples of good reporting with checklist of items to include when reporting a randomized trial and the flow of participants through each stage of a randomized trial.

Figure 4.1: Revised template of the CONSORT (Consolidated Standards of Reporting Trials) diagram showing the flow of participants through each stage of a randomized trial (Curtsey of Douglas *et al.*, 2001).

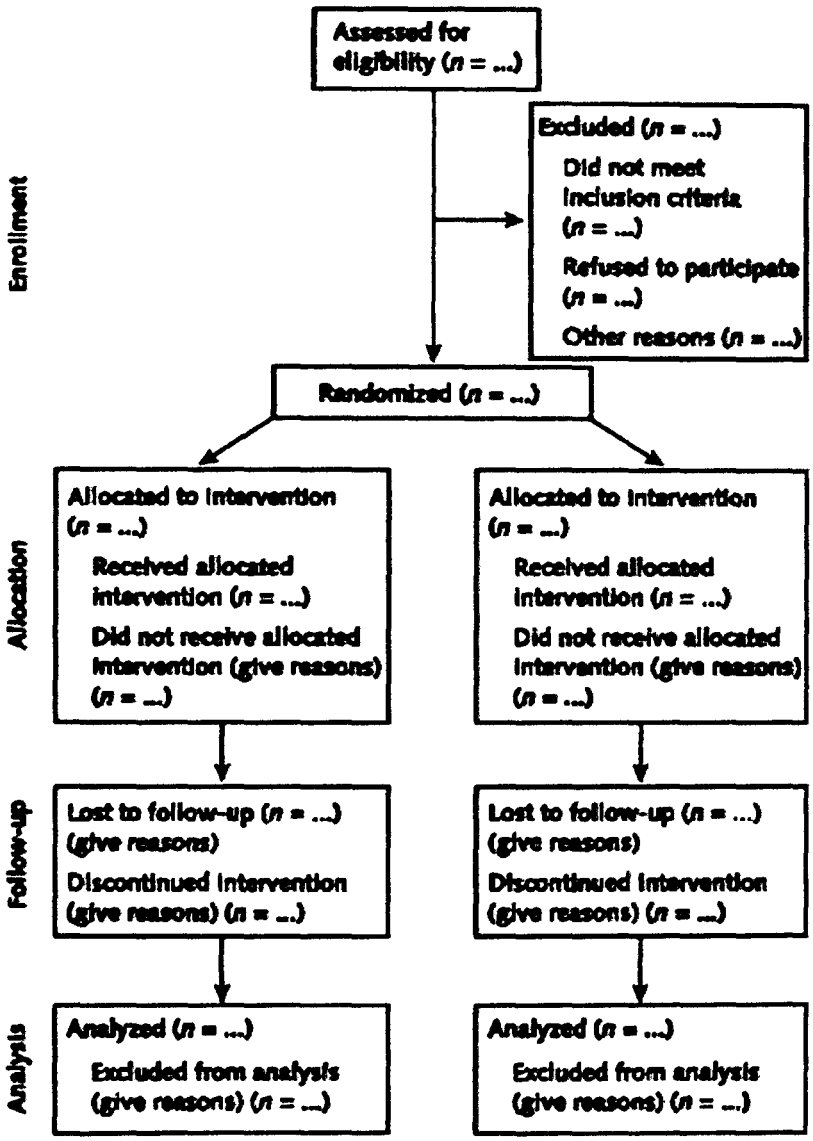


Table 4.1: Checklist of Items to Include When Reporting a Randomized Trial (Curtsey of Douglas *et al.*, 2001).

Paper Section and Topic	Item Number	Descriptor	Reported on Page Number
Title and abstract	1	How participants were allocated to interventions (e.g., "random allocation," "randomized," or "randomly assigned").	
Introduction			
Background	2	Scientific background and explanation of rationale.	
Methods			
Participants	3	Eligibility criteria for participants and the settings and locations where the data were collected.	
Interventions	4	Precise details of the interventions intended for each group and how and when they were actually administered.	
Objectives	5	Specific objectives and hypotheses.	
Outcomes	6	Clearly defined primary and secondary outcome measures and, when applicable, any methods used to enhance the quality of measurements (e.g., multiple observations, training of assessors).	
Sample size	7	How sample size was determined and, when applicable, explanation of any interim analyses and stopping rules.	
Randomisation	8	Method used to generate the random allocation sequence, including details of any restriction (e.g., blocking, stratification).	
Sequence generation	9	Method used to implement the random allocation sequence (e.g., numbered containers or central telephone), clarifying whether the sequence was concealed until interventions were assigned.	
Allocation concealment Implementation	10	Who generated the allocation sequence, who enrolled participants, and who assigned participants to their groups.	
Blinding (masking)	11	Whether or not participants, those administering the interventions, and those assessing the outcomes were blinded to group assignment. If done, how the success of blinding was evaluated.	
Statistical methods	12	Statistical methods used to compare groups for primary outcome(s); methods for additional analyses, such as subgroup analyses and adjusted analyses.	
Results			
Participant flow	13	Flow of participants through each stage (a diagram is strongly recommended). Specifically, for each group report the numbers of participants randomly assigned, receiving intended treatment, completing the study protocol, and analyzed for the primary outcome. Describe protocol deviations from study as planned, together with reasons.	
Recruitment	14	Dates defining the periods of recruitment and follow-up.	
Baseline data	15	Baseline demographic and clinical characteristics of each group.	
Numbers analysed	16	Number of participants (denominator) in each group included in each analysis and whether the analysis was by "intention to treat." State the results in absolute numbers when feasible (e.g., 10 of 20, not 50%).	
Outcomes and estimation	17	For each primary and secondary outcome, a summary of results for each group and the estimated effect size and its precision (e.g., 95% confidence interval).	
Ancillary analyses	18	Address multiplicity by reporting any other analyses performed, including subgroup analyses and adjusted analyses, indicating those prespecified and those exploratory.	
Adverse events	19	All important adverse events or side effects in each intervention group.	
Discussion			
Interpretation	20	Interpretation of the results, taking into account study hypotheses, sources of potential bias or imprecision, and the dangers associated with multiplicity of analyses and outcomes.	
Generalisability	21	Generalisability (external validity) of the trial findings.	
Overall evidence	22	General interpretation of the results in the context of current evidence.	

4.2 Materials and Methods

4.2.1 Objectives

4.2.2 Primary Objective:

To evaluate the effects of 1426 ppm fluoridated toothpaste on surface loss progression of human enamel and dentine compared to a placebo in an experimental in situ longitudinal erosion model. The null hypothesis was that there is no difference between 1426 ppm fluoridated toothpaste and placebo on tooth surface loss of enamel and dentine in an erosive challenge *in situ*.

4.2.3 Secondary Objective

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

4.2.4 Study Design

This was a randomised, double-blind cross-over study. The study involved two study arms, each to test one of the two test products. The length of each study arm was one month making a total of two months for the whole study.

Participants dipped their upper removable appliances in citric acid extra-orally five times daily for two minutes each time and rinsed their devices with one of the two toothpastes twice a day. This procedure was repeated for 21 days in each study arm.

The amount of surface loss was calculated at the end of each study arm using light surface profilometry (Proscan Scantron) which measured the depth of the eroded surface compared to the intact surface. The amount of surface loss was calculated by using the automated software supplied with the surface profilometer.

4.2.5 Ethical and Regulatory Aspects

Ethical approval for this study was sought from the Research Ethics Committee of Leeds Health Care/ United Leeds Teaching Hospital Trust and Research and Development

Department at University of Leeds (Appendix 38 and 39). The Principal Investigator ensured that this study was conducted in full conformance with the laws and regulations of the country in which the research was conducted and the Declaration of Helsinki/Venice/Tokyo/Hong Kong/South Africa (1996).

4.2.6 Study Population and Power Calculation

Study population was calculated using the standard deviation was found in our *in vitro* study (study-6) and a mean difference of 5 μm. The power calculation was set for 90% for enamel and 80% for dentine. This is due to the difference in sample number required when power calculation was set to 90% for both enamel and dentine. It was thought that 30 subjects were a reasonable estimate in order to yield sufficient accurate data.

Forty-four volunteers were screened and 37 subjects were randomised to ensure that at least 30 subjects completed the study. The age range of the volunteers was 18-65 years. Volunteers were mainly from the staff at Leeds Dental Institute or students at Leeds University.

Volunteers were employed from the students or staff at Leeds University or LGI. Information sheets (Appendix 40) were distributed and a signed informed consent (Appendix 41) was obtained prior to recruitment of volunteers into the study. They were given a dental examination before the start of the study to determine their DMFT/DMFS using BASCoD criteria (Mitropoulos *et al.*, 1992). Forty-four volunteers were screened at the beginning of the study, and 37 volunteers were randomised to enable 30 subjects to complete the study.

4.2.6.1 Inclusion Criteria

1. Adults with normal salivary function who were not taking medications that could affect 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 0.25 ml/min was required for participation in the study.
2. Minimum of 18 natural teeth.

3. Free from clinical signs of periodontal disease.
4. Be able to comply with the protocol instructions.
5. Medical history did not include any medical contraindications 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. Were 18 to 78 years old and in good general health with no evidence of communicable diseases;
8. Had an unstimulated whole salivary flow rate ≥0.2 ml/min and a stimulated whole salivary flow rate ≥0.8 ml/min;
9. were able to wear the appliances as required by the protocol
10. Were able to comply with the experimental procedures.

4.2.6.2 Exclusion criteria

1. Signed informed consent not obtained by the volunteers.
2. Adults taking drugs that could have affected the saliva 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 four weeks.
6. Volunteers who had antimicrobial treatment in the previous two 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. Any medical condition that could have been expected to interfere with the subject's safety during the study period
10. Having taken any medication that could have potentially reacted with the test products;
11. That required antibiotics prior to dental treatment
12. Demonstrated an inability to comply with the study procedures.
13. Volunteers that showed signs of moderate or severe tooth wear.

4.2.6.3 Subject Withdrawal Criteria

Subjects had the right to withdraw from the study at any time and for any reason. The investigator also had the right to withdraw subjects from the study in the event of inter-current 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 render the study underpowered; therefore, unnecessary withdrawal of subjects should be avoided. Should a subject decide to withdraw, all efforts were made to complete and report the observations as thoroughly as possible. A complete final evaluation at the time of the subject's withdrawal was 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 was also recorded on the case report form '(CRF)'. A description of the 'stopping rules' or 'discontinuation criteria' for individual subjects were described.

4.2.7 Study Products

4.2.7.1 Test Product

Sensodyne ProNamel® 1426 ppm Fluoride + 5% potassium nitrate.

4.2.7.2 Reference Product

0 ppm Fluoride Pronamel (Placebo).

4.2.8 Study Duration and Timings

As described above, the two study arms were for 30 days each.

4.2.9 Criteria for Evaluation

4.2.10 Efficacy

4.2.10.1 Surface Loss

Our preliminary work showed that measurement of the amount of surface loss was an ideal technique to compare the effects of different preventive toothpastes. Therefore, the mean values of surface loss were used as a method of comparison. The amount of surface loss was calculated using light surface profilometry (Proscan Scantron) which measured the depth of the eroded surface compared to the intact surface. The amount of surface loss was calculated by using the automated software supplied with the surface profilometer. The difference between treatment groups was compared. A 5 μm difference in the surface loss between the two treatment groups was considered as significant difference.

4.2.10.2 Safety

Treatments were compared for the number of subjects with oral soft tissue adverse events (AEs).

4.2.11 Test Methods

4.2.11.1 Surface Profilometery (SP)

SP gives an idea of the surface profile of the slabs that were used in our study. In addition, it measured the depth of surface loss. Therefore, SP was used at the beginning of the study to ensure that the slabs' surface was flat and at the end of each leg of the study to measure the depth of surface loss. Technique was described at section 3.2.2.3 (page 67).

4.2.11.2 Knoop microhardness (KMH)

KMH was used as an inclusion criterion for enamel and dentine slabs. 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. The technique was described in section2.5.1 (page 28).

4.2.12 Slab Preparation

This part was explained previously (sections 2.4.1 on page 25; 3.2.2.1 on page 67; and 3.3.2.4 on page 78).

4.2.13 Storage of Slabs

Once the slabs had been prepared, they were kept moist in de-ionised distilled water in micro-centrifuge tubes and left at room temperature preparing them to be sterilised. This process was repeated after finishing the experiment.

4.2.14 Sterilisation and Storage of Slabs

The slabs were stored damp in sealed containers and exposed to gamma radiation (4080 Gy). 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 (Amaechi *et al.*, 1998b). Then slabs were immersed in 5% sodium hypochlorite for 24 hours to eliminate prions. 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 up to the date of analysis. The slabs were handled at all time using disposable medical gloves.

4.2.15 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. Four slabs, two enamel slabs and two dentine slabs were secured in the palatal plate of the appliance. The slabs were assigned to the side of the midline and secured with sticky wax; care was taken to ensure that the wax did not cover the exposed surface of the slabs. Slabs were exposed to the oral environment but they were protected from the effect of the tongue using arched wires leaving a space of 1 mm between the wire and the slabs (Figure 4.2 to Figure 4.5).

Figure 4.2: Shows the removable denture used in the study.

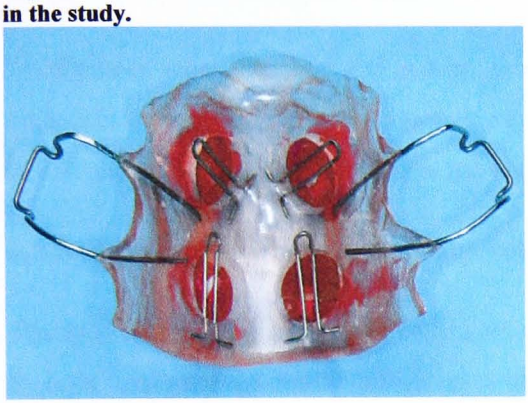


Figure 4.3: The removable denture intra-orally.

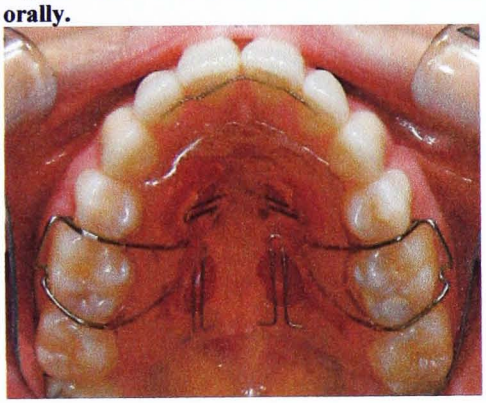


Figure 4.4: Shows the removable upper appliance with 4 holes covered with two arched wires each to prevent the effect of the tongue on the slabs. Two holes were used for enamel and the other two for dentine slabs.

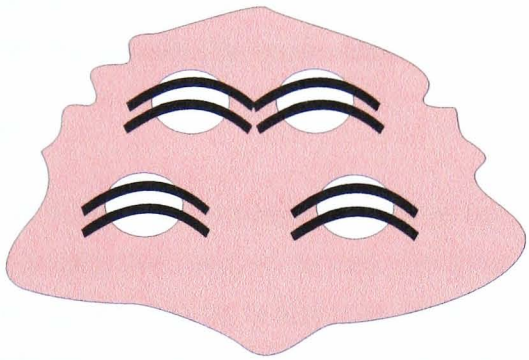
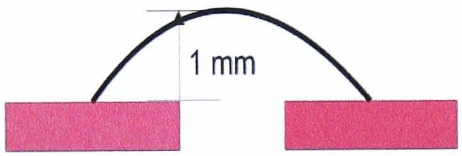


Figure 4.5: Shows the orientation of the wire over the whole made in the denture.



4.2.16 Blindness and Randomisation

4.2.16.1 Randomisation

Following the baseline evaluations, the subjects that met all of the eligibility criteria were randomized. Subjects were given one of each of the two treatments during each study period according to a randomisation schedule (Appendix 42).

4.2.16.2 Blindness

Toothpastes were matched for colour, smell, taste, and containers. In addition, the test materials were coded and the codes were kept with the study sponsor. Neither the principal investigator nor the volunteers knew the codes of the test materials during the study.

4.2.16.3 Rules for Breaking the Study Blindness

The blindness of the study was only allowed to be broken in an emergency where it was thought essential to know which treatment a subject had received in order to give appropriate medical care. The investigator had to sign and date the broken code envelope and give the reason for breaking the code. This was not required during this study as there was no serious adverse event.

4.2.17 Study Procedures and Assessments

4.2.17.1 Informed consent

An e-mail with an advert was sent to potential participants. Interested participants received an information sheet and they were given a minimum period of seven days before being invited to the first visit. Prior to commencement of any study-related activity, the investigator obtained written (signed and dated by the subject) informed consent from each individual participating in this study following an adequate explanation of the aims, methods, objectives and potential hazards of the study. The investigator also explained to the subjects that they were completely free to refuse to enter the study or to withdraw from it at any time. The investigator noted the date and time of the consent in the subject's records. A subject was considered to be enrolled into the study after the informed consent was signed and witnessed.

4.2.17.2 Screening

A subject screening record and a CRF were used to document the screening evaluation along with any reason for failure (Appendix 43).

At the screening visit, eligible subjects were randomised to receive 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: The investigator recorded each subject's date of birth, gender and race in the CRF.
2. Medical History: The investigator (or medically qualified designee) took the medical history of each subject including details of any relevant medical or surgical history, allergies or drug sensitivities. 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 designee. Both current and concomitant medications were restricted in accordance with the exclusion criteria.
3. 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. The results of the oral examination were recorded in the CRF as either normal or abnormal with any abnormalities being described.
4. Salivary flow rate: A referenced salivary flow rate (an unstimulated whole salivary flow rate $\geq$ 0.2 ml/min and a stimulated whole salivary flow rate $\geq$ 0.8 ml/min) was measured to ensure that a standard remineralisation effect of the saliva of all volunteers was achieved. The subjects were seated in a quiet, comfortable position, with their head tilted forward so that saliva collected in 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 five minute saliva collection period. During this five minute period the subject was not permitted to swallow any saliva but was 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 for one minute. After one minute the, subjects were instructed to swallow any pooled saliva. They then chewed the gum base for two minutes during which time they emptied any pooled saliva into a collection tube.

During the saliva collection period the subjects were not permitted to drink, chew or speak. An audible alarm sounded after five 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.

5. Measurements made for *in-situ* oral appliances: Subjects were seated in a comfortable position in a dental chair. In order to protect clothing, subjects wore 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 and placed in the subject's mouth to give an impression of the upper and lower jaw/arch. Natural bite was recorded in wax. The impressions and wax bite were disinfected before being transported to the lab.

4.2.17.3 Treatment Phase

This section was summarized with a flow chart (Table 4.2 and Table 4.3 on page 169 and page 170).

4.2.17.4 Washout Period

A washout period of at least seven days prior to the start of study arm **one** was commenced after the intra-oral device fitting visit (visit-2). Subjects were provided with a standard toothbrush and fluoride-free toothpaste at this visit. The washout period between study legs also had a duration of at least seven days.

During the washout periods, the subjects were only permitted to use the standard toothbrush and the non-fluoride (Boots non-fluoride toothpaste, England, the UK), non-

xylitol containing toothpaste supplied and abstained from all oral hygiene procedures (flossing and using a breath freshener or mouth wash etc).

At the end of the washout period prior to commencing study arm one subjects returned their *in-situ* oral appliances to the study site so that they were disinfected and fitted with four slabs (two enamel and two dentine slabs). At the end of each study arm, the subjects left their *in-situ* intra oral appliances at the study site for four fresh slabs to be inserted into their appliances in preparation for the next study arm.

4.2.17.5 Acclimatisation Period

Prolonged use of the *in-situ* oral appliances could potentially cause some discomfort to the subjects. Hence, a try-in period with the *in-situ* oral appliance of 2-7 days was conducted prior to commencement of study arm one (i.e. concurrent with the washout phase of study arm one). During this period the subjects tried to wear their appliances at all times (except when eating, drinking, or brushing their teeth).

For this study any product put into the oral cavity, including chewing gum, flossing or using a breath freshener or mouthwash was considered as eating and drinking. If the subject experienced discomfort, they were asked to return to the study site for the appliance to be adjusted. They then continued with the acclimatisation period.

4.2.17.6 Follow-up period

Subjects attended for a follow-up visit within 14 days of the final assessment day. The visit included a brief medical interview, oral examination and optional application of commercially available topical fluoride gel or remineralisation treatment (tooth mousse/Duraphat).

4.2.18 Experimental Protocol/ Regime

The subjects were assigned to one of the two test regimes using specially designed appliances with fixed enamel and dentine slabs. The regime was as follow:

1st Period	1 week	Wash-in before starting the 1st period
	3 weeks	Dipping the appliance twice a day, morning and evening into toothpaste which has fluoride concentration of either 1426 ppm F as NaF or 0 ppm F. During the day, the patient dipped the appliance 5 times into 0.3% citric acid (pH 3.6). Dipping time was 2 minutes.
2nd Period	1 week	Wash-out before starting the 2nd period
	3 weeks	Dipping the appliance twice a day, morning and evening into toothpaste which has fluoride concentration of either 1426 ppm F as NaF or 0 ppm F. During the day, the patient will dip the appliance 5 times into 0.3% citric acid (pH 3.6). Dipping time was 2 minutes.

Volunteers were given supplies for one week at a time. Therefore, volunteers attended the study site for each study arm as follow:

1. 1st Visit: Appliance fitting
2. 2nd Visit (at day 2): to check the appliance.
3. 3rd Visit (at day 7): to give supplies for 1st week and to check the appliance.
4. 4th Visit (at day 14): to give supplies for 2nd week.
5. 5th Visit (at day 21): to give supplies for the 3rd week.
6. 6th Visit (at day 29): to collect dipping diary and appliance.

The appliances were worn continuously by the volunteers, except at mealtimes, whilst drinking, or during tooth brushing. Dipping into toothpaste was achieved by asking the volunteer to brush for one minute using the toothpaste supplied as normal and while the appliance was not worn. Then the volunteers inserted their appliances before rinsing with water and they were asked to swish using the toothpaste in their mouth for one minute. After that, volunteers rinsed with water as normal. The enamel and dentine slabs were collected at the end of each period and the volunteers were supplied with new slabs. The volunteers used fluoride-free toothpaste provided for them, which they used twice daily. The study lasted three months.

4.2.19 Compliance

The volunteers' compliance was checked using the following methods:

1. Each volunteer had a case record form to monitor and record each step throughout the study.
2. Dipping diary which was checked every week at volunteer visits (appendix 44).
3. The used citric acid bottles were collected and the remnants of each bottle were measured and recorded on the product disposal form.
4. Used toothpaste tubes were collected and the used amount was weighed.
5. Used toothbrushes were collected after each study arm and their bristles were checked.

Table 4.2: Flow Chart

RECRUITMENT

↓

SCREENING

(SALIVARY FLOW, DMFS SCORE)

↓

PREPARATION OF IN SITU DEVICES

↓

WASH-IN PERIOD (1 WEEK)

↓

PRODUCT USE "BRUSHING TWICE DAILY" (21 DAYS) AND 2 MINUTE DIPPINGS OF THE SLABS IN CITRIC ACID 5 TIMES PER DAY AS PER INSTRUCTION SHEET. THE SUBJECTS RECORD THE EXACT TIMES OF DIPPING ON A DIARY CARD

↓

THE SLABS REMOVED AT 21 DAYS OF CYCLING (THE SLABS TESTED WITH SURFACE PROFILOMETRY)

↓

WASH-OUT PERIOD (1 WEEK)

↓

REPEATED WITH 2ND TEST PRODUCTS

Table 4.3: Study Schedule

Steps	V1 Screen	V2 Day 0*	V3 Day 2**	V4 Day 7 (Treatment Start- 1st Leg)	V5 Day 14	V6 Day 21	V7 Day 29 (Treatment End- 1st Leg)	V8 Day 36 (Treatment Start- 2nd Leg)	V9 Day 41	V10 Day 48	V11 Day 56 (Treatment End- 2nd Leg)
Consent	X										
Medical History	X										
Demographics	X										
Concurrent Medications	X										
Inclusion/Exclusion	X										
Oral Soft and Hard Tissue	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
Randomisation	X										
Partial Denture		X	X	X				X			
Distribute Washout		X									
Collect Washout				X				X			
Place Slabs				X				X			
Collect Slabs							X				X
Randomisation		X									
Distribute Product/Diary				X	X	X		X	X	X	
Collect Product/Diary					X	X	X		X	X	X
Supervise Brushing		X	X	X				X			
Non Treatment Events	X	X	X								
Adverse Events			X	X	X	X	X	X	X	X	X
Prophylaxis											X

***Upper Removable Intra-Oral Appliance fitting for first time**

****** **Upper Removable Intra-Oral Appliance check visit**

4.2.20 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.

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 have been 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 have been 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 the clinical trial after treatment had started, whether considered related to treatment or not. "Treatment" included 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).

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 having not interfered with normal everyday activities.

MODERATE - sufficiently discomforting to have interfered with normal everyday activities.

SEVERE - incapacitating and/or prevented 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 repeated 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 appropriate.

Any adverse events ongoing at the follow-up visit, which had any association with the study medication or the study regime was followed-up until resolved by the study site and for two weeks after the subject's last visit., if resolution did not occur sooner. Any resolutions confirmed by the study site were noted in the study file.

4.2.20.1 Serious Adverse Events

Any clinical adverse event, including abnormal laboratory test value, that was serious (as defined below) and occurred during the course of the study, irrespective of the treatment received by the subject, was reported to the study sponsor and ethics 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 resulted in any of the following outcomes:

DEATH

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

PERSISTENT OR SIGNIFICANT DISABILITY/INCAPACITY (disability was 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 might be considered a serious adverse experience when, based upon appropriate medical judgment, they might have jeopardised the patient or subject and may have required medical or surgical intervention to prevent one of the outcomes listed in the list of definitions.

The terms "cancer" and "overdose" were not part of the definition of a serious AE, but if a patient experienced cancer it was still reportable as a serious AE.

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

The term 'severe' was a measure of intensity; thus a severe adverse event was not necessarily serious. For example, nausea of several hours duration might have been rated as severe, but might have not been clinically serious.

A death occurring during the study or which came to the attention of the investigator within four weeks after stopping the treatment whether considered treatment-related or not had to be reported.

Serious adverse events 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 had to be recorded on the clinical study Serious Adverse Event Form.

If there was no reply, the details had to be recorded on the message service.

Such preliminary reports were to be followed by detailed descriptions later which would have included copies of hospital case reports, autopsy reports and other documents when requested and applicable.

The Ethics Committee was to be notified of such an event in writing as soon as was practical.

4.2.21 Study Treatment Supplies Management

Test products were matched except for the amount of fluoride content.

1. 1425pm F as NaF toothpaste (Sensodyne Pronamel®) as a test product.
2. 0 ppm F toothpaste as a placebo.
3. In addition, volunteers were supplied with pre-weighed bottles of citric acid and bottles of Volvic water to produce fresh erosive solutions every day.

The only slight risk expected due to the use of fluoride free toothpaste (0 ppm F) was a very small chance of enamel mineral loss. This is because of the short period (5 weeks in total) that subjects had to use it. However, teeth regain lost minerals naturally by saliva. As an extra precaution, a topical fluoride gel (duraphate) was applied at the end of the study to each volunteer. These information were provided in the ethical submission to ethical committee and were highlited in the volunteers' information sheet.

4.2.21.1 Packaging and labeling

Volunteers were supplied with 100 ml of all toothpastes to standardise the amount. Toothpastes were packaged with white covers and labelled using codes. All study labels included at least the following information: Protocol number, randomisation and period number, storage conditions, and emergency contact details. A Sensodyne Pronamel toothbrush was supplied to each volunteer at the beginning of each study arm. Citric acid crystals were supplied in plastic bottles. The weight of citric acid in each bottle was enough for one day. Another glass bottle was supplied to mix the bottled water and citric acid crystals. The glass bottle had an indication line at the level for water to be added. In addition, volunteers had a dipping pot to dip the removable appliances extra-orally in citric acid. This pot had an indication line to standardise the amount of erosive citric acid used for each dipping.

Packaging and labeling of all study products was carried out according to the International Conference on Harmonisation Good Clinical Practice (ICH GCP) guidelines and was the responsibility of the Clinical Supply Department, GSKCH, Weybridge, UK and the principal investigator at Leeds Dental Institute.

4.2.21.2 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 was dispensed.
2. Date and quantity of the study treatment dispensed to the subject.
3. Date and quantity of the study treatment returned by the subject (if applicable).

An inventory was carefully maintained by the investigators during the study:

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

4.2.22 Monitoring of the Study

Study site personnel monitored the study whilst maintaining subject confidentiality.

It was the study site's responsibility to inspect the CRFs at regular intervals throughout the study to verify the adherence to the protocol and the completeness, consistency, and accuracy of the data being entered.

4.2.23 Study Documentation, CRFs, and Record Keeping

4.2.23.1 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.

The investigator's study master file contained the protocol/amendments, case report and query forms, IRB/IEC and governmental approval with correspondence, informed consent, drug records, staff curriculum vitae, and authorisation forms and other appropriate documents/correspondence, etc.

Subject/patient clinical source documents (defined in advance to record key efficacy/safety parameters independent of the CRFs) included subject/patient hospital/clinic records, physician's and nurse's notes, appointment book, original laboratory reports, ECG, EEG, X-ray, pathology and special assessment reports, consultant letters, screening and enrolment logs, etc. These two categories of documents were kept on file by the investigator according to local regulations.

4.2.24 Confidentiality of Study Documents and Subject Records

The investigator assured that the subject's anonymity was maintained. On CRFs or other documents subjects were not identified by their names, but by an identification code.

The investigator kept a separate log of subjects' codes, names and addresses in a master file kept by Professor MS Duggal.

4.2.25 Statistics and Data handling

More details of the proposed statistical analysis were documented in the statistical analysis plan, which was written following finalisation of the protocol and prior to the database lockout and analysis

4.2.25.1 Demographic and baseline characteristics

Information of randomized subjects, number of valuable subjects, age, gender and race was collected. The amount of tooth surface loss (hard tissue findings) was collected. Descriptive statistics for continuous measures included the number of subjects, mean, median, standard deviations and minimum/maximum.

Data were entered manually when microhardness or profilometry measurements were made. About 10% of the data were re-analysed randomly by one of the study supervisors (Dr. S Strafford) to validate the data. The data were then transferred onto spreadsheets. Approximately 10% of the data were rechecked by Dr. S Strafford to validate the data entry. SPSS statistical software was used to analyse the data at the end of the study.

4.2.25.2 Efficacy

The primary efficacy variable was the mean tooth surface loss (enamel and dentine) as measured by profilometry in micrometers after 21 days of study treatment for the per protocol population.

Each of the primary efficacy variables were analyzed separately using ANOVA appropriate for a cross-over design. The model included the following: factors subject, treatment, period, salivary flow rate (as continuous) and side of mouth treated (left/right) as covariates. The subject was included as a random effect.

Mean differences between treatments and their 95% confidence intervals were presented. The assumptions of the analysis were explored using appropriate methods when testing the assumptions of normality and constancy of variance. When any of the assumptions were violated an appropriate data transformation or non-parametric analysis was used accordingly.

AEs were regarded as 'non treatment emergent' when they occurred between screening and the time of administering the first treatment application. All other AEs were considered to be 'treatment emergent'. Treatment emergent AEs were listed only.

Oral soft tissue results (abnormal/normal), oral AEs, and concomitant medications were also listed.

4.2.25.3 Primary efficacy variables

The mean tooth surface loss was calculated using the automated software of profilometry within each enamel block. The comparisons between the two test products were conducted using paired t-tests. The t-test was two-sided and the significance level was 0.05. No adjustment for the alpha-level was planned.

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 consideration of our previous *in vitro* results. In addition, the possibility of remineralisation of dental slabs in the oral environment was considered. The power calculation was set to be 90%.

4.3 Results

The results will be presented in accordance to CONSORT statement (Begg *et al.*, 1996).

4.3.1 Number of subjects (planned and analysed)

In the randomised population of 40 subjects, 28 were females and 12 were males. The mean age was 34.3 years (range 20.0-58.0). Two of these subjects did not receive any of the study products and one subject commenced period one but failed to attend for any further study visits (Appendix 45 & 46).

The intention to treat (ITT) population contained 37 subjects and both treatment groups (as defined by sequence of administration of products) appeared to be well balanced in terms of age and salivary flow rate. In this population the mean age was 35.2 years (range 21.0-58.0) with 26 female subjects and 11 male subjects. There was some imbalance with regard to sex (predominance of females in the placebo first group) although with such small numbers randomised; this was not regarded as unusual. The per-protocol population contained 35 subjects. I presented results, ITT and per-protocol populations, as matter of comparison in the rest of the result section.

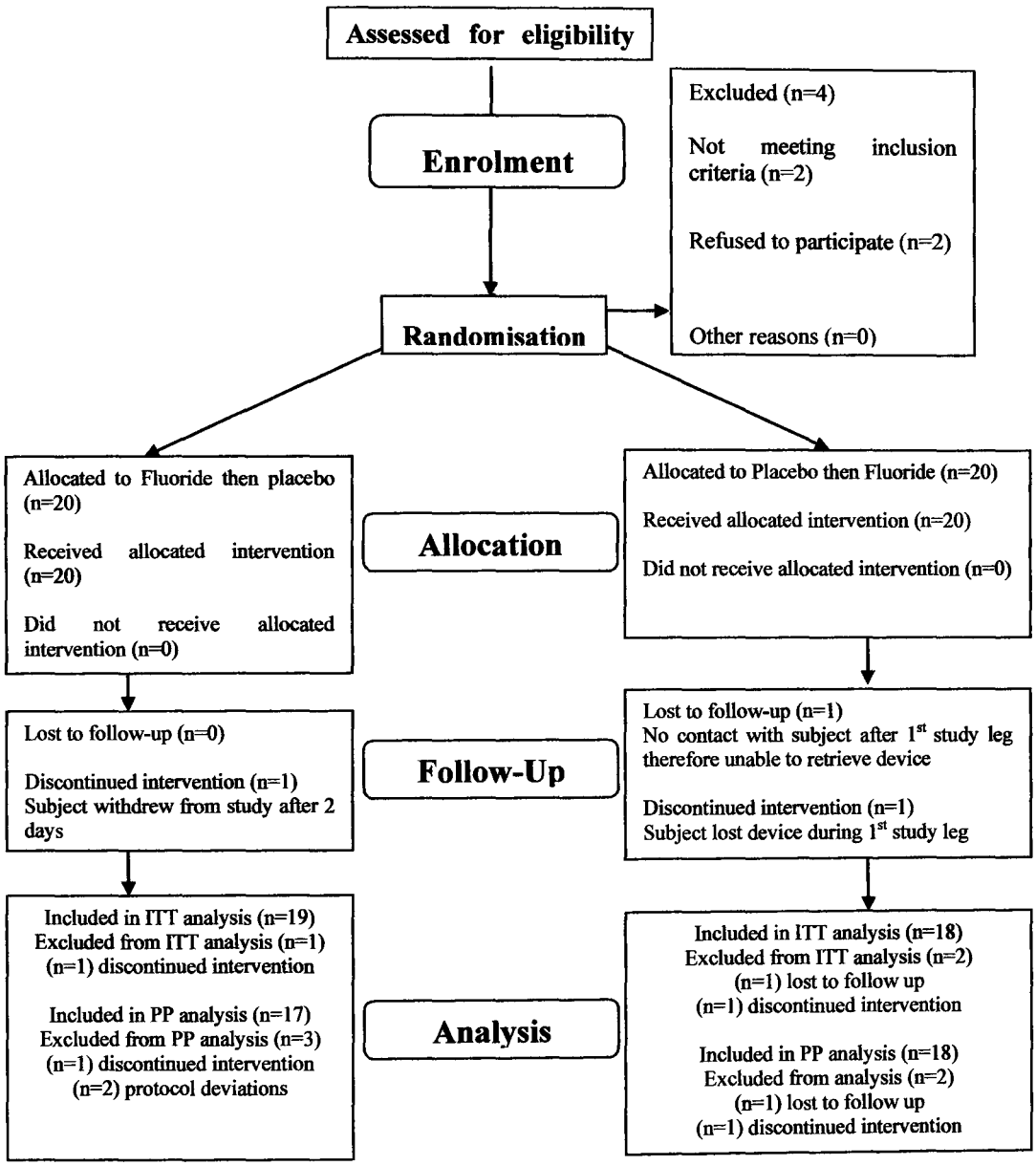
4.3.2 Safety Results

Three adverse events were recorded during the study, two of gingivitis and one of gingivitis with a broken filling. None of the adverse events were thought to be treatment related as the cases of gingival infection occurred during treatment with placebo and during the washout period between study legs whilst the case of gingival infection with a broken filling occurred during treatment with fluoride.

4.3.3 Demographic Characteristics

Table 4.4 summarises the demographic characteristics.

Table 4.4: Consort Diagram.



4.3.4 Efficacy Results (Summary of Enamel and Dentine results)

Analysing the ITT population (Appendices 47 to Appendix 50) showed that the distribution of both enamel surface loss and dentine surface loss was very skewed and that the standard deviations were much larger than was anticipated in the sample size calculations.

Examining the mean surface loss from at Day 21 and the ratio of geometric means (Table 4.5, Table 4.6, Figure 4.6, and Figure 4.7) showed that less than half as much erosion occurred for both enamel and dentine when Sensodyne ProNamel® 1426 ppm Fluoride + 5% potassium nitrate was used compared to the placebo.

In addition, these tables shows amount of tooth surface loss of enamel was less than the amount of surface loss of dentine.

Table 4.5: Summary of the mean surface loss (µm) of enamel and dentine at day 21 (ITT population)

Treatment	Enamel	Dentine
Pronamel	11.45	16.01
Placebo	25.29	29.20

Table 4.6: Day 21. Ratio of geometric means surface erosion between Sensodyne ProNamel® 1426 ppm Fluoride + 5% potassium nitrate and placebo

Treatment	Ratio of geometric means† (Fluoride VS placebo)	95% CI	P-value
Enamel	0.41	0.33 to 0.52	<0.001*
Dentine	0.43	0.34 to 0.54	<0.001*

† Ratio of means less than 1 indicated less erosion with Sensodyne ProNamel® 1426 ppm Fluoride + 5% potassium nitrate than with placebo. * Statistically significant result

Figure 4.6: Summary of Enamel surface loss (µm) from baseline to Day 21 (Intention to Treat Population). Bars represent standard deviations.

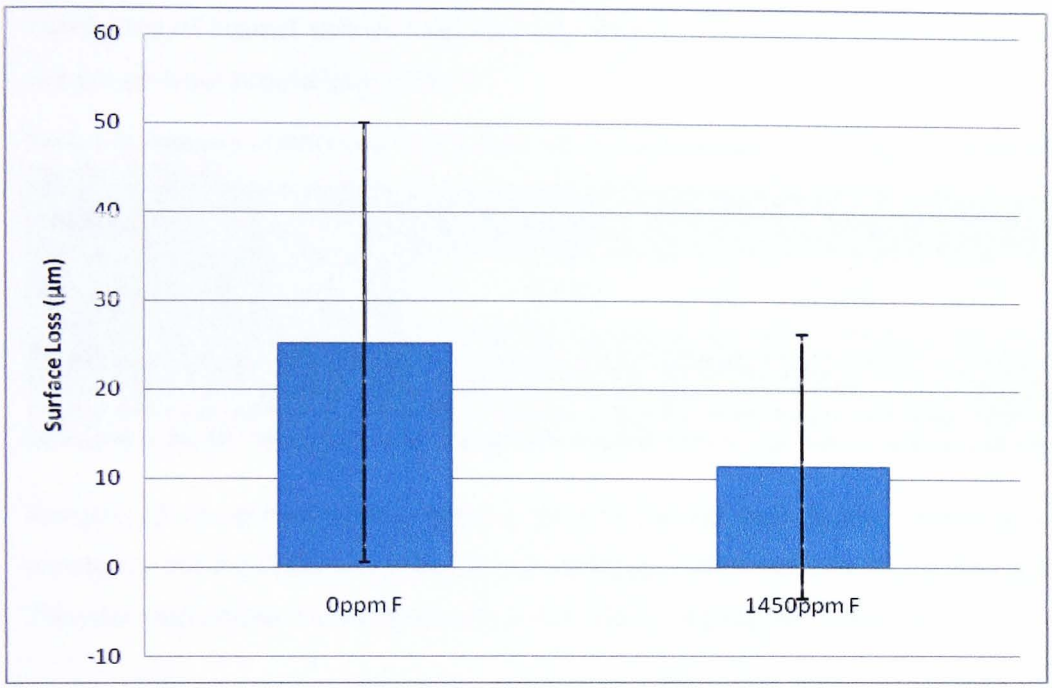
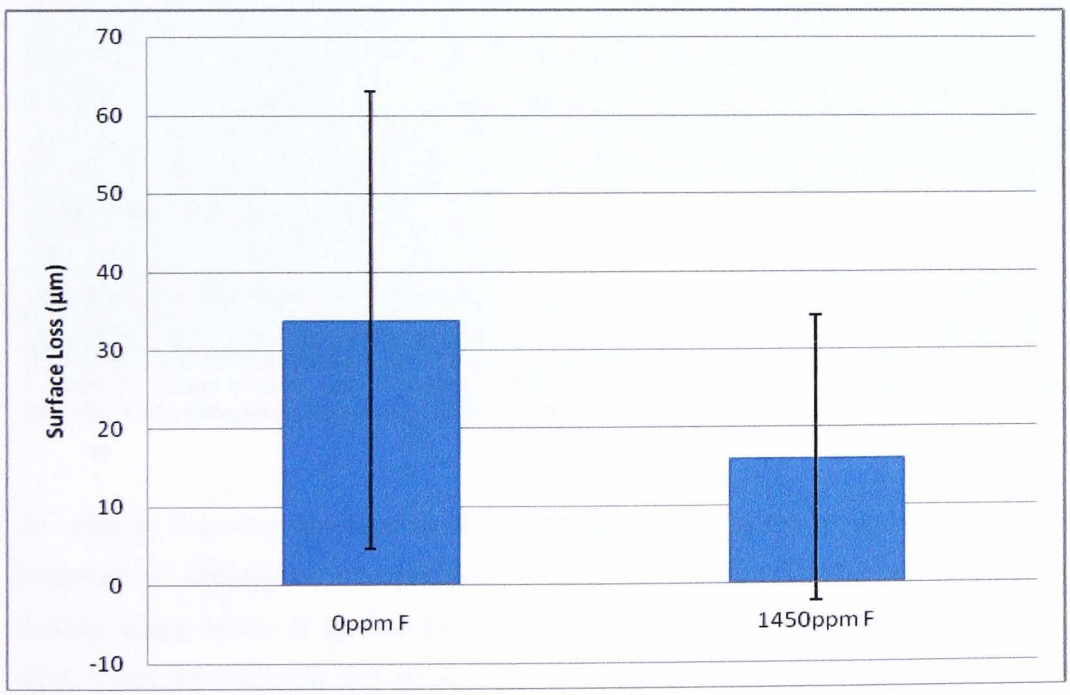


Figure 4.7: Summary of Dentine surface loss (µm) from baseline to Day 21 (Intention to Treat Population). Bars represent standard deviations.



4.3.5 Detailed Results for Enamel Surface Loss

Natural log of the amount of surface loss was used in the primary analysis as the distribution of enamel surface loss was very skewed. The analysis included both ITT and per-protocol populations (Table 4.7).

Table 4.7: Summary of difference in log surface loss of dental enamel (Natural log µm, Fluoride VS Placebo) at Day 21.

log Enamel	N	Mean	Std.	Min	Max
ITT population	37	-0.886	0.683	-2.429	0.177
Per-Protocol population	35	-0.938	0.664	-2.429	0.088

Subject difference calculated as Fluoride – Placebo. Negative differences for the mean difference indicate that the first formulation (Fluoride) resulted in less surface loss. Data were extracted from

Analysis of the per-protocol population (n=35) yielded very similar results as ITT population and the difference in favour of Sensodyne ProNamel® 1426 ppm Fluoride + 5% potassium nitrate. This difference was statistically significant (Table 4.8).

Table 4.8: Results of enamel primary efficacy (tooth surface loss). Ratio of geometric mean surface loss between Sensodyne ProNamel® 1426 ppm Fluoride + 5% potassium nitrate and placebo at day 21.

	Ratio of geometric means† (Fluoride VS Placebo)	95% CI	P-value
Enamel (ITT Population)l	0.41	0.33 to 0.52	<0.001
Enamel (Per-Protocol population)	0.39	0.33 to 0.52	<0.001

† Ratio of means smaller than 1 indicates less erosion with Sensodyne ProNamel® 1426 ppm Fluoride + 5% potassium nitrate than with placebo.

In addition there also appeared to be an imbalance between the groups (as defined by sequence of administration of products) with those subjects administered fluoride first having higher levels of surface loss in both periods than those administered placebo first. Table 4.9 indicated that surface loss was greater during the placebo period than during the fluoride period for enamel.

Table 4.9: Summary of Enamel surface loss (µm) at day 21 (Intention to Treat Population). First group shows results of subjects who started fluoride treatment first. Second group shows results of subjects who started placebo treatment first.

Group	variable	N	Mean	SD	max	min
Pronamel/Placebo	Pronamel	19	14.48	17.77	82.63	1.83
	Placebo	19	30.18	28.08	122.21	3.05
Placebo/Pronamel	Pronamel	18	8.24	10.79	46.63	1.72
	Placebo	18	20.12	20.08	74.59	2.34
Total	Pronamel	37	11.45	14.93	82.63	1.72
	Placebo	37	25.29	24.71	122.21	2.34

4.3.6 Detailed Results for Dentine Surface Loss

As for enamel the data were logged for using SPSS because of the skewed standard deviations before it was analysed. The analysis included both ITT and per-protocol populations (Table 4.10).

Table 4.10: Summary of difference in log surface loss of dental dentine (Natural log µm, Fluoride VS Placebo) at Day 21.

log Dentine	N	Mean	SD	Min	Max
ITT population	37	-0.848	0.672	-2.046	0.041
Per-Protocol population	35	-0.898	0.656	-2.046	0.010

Subject difference calculated as Fluoride – Placebo. Negative differences for the mean difference indicate that the first formulation (Fluoride) resulted in less surface loss.

Again, Analysis of the per-protocol population (n=35) yielded very similar results as ITT population and the difference in favour of Sensodyne ProNamel® 1426 ppm Fluoride + 5% potassium nitrate. This difference was statistically significant (

Table 4.11).

Table 4.11: Ratio of geometric means surface loss of dentine between Sensodyne ProNamel® 1426 ppm Fluoride + 5% potassium nitrate and placebo at day 21.

	Ratio of geometric means† (Fluoride VS Placebo)	95% CI	P-value
Dentine (Intention to Treat Population)l	0.43	0.34 to 0.54	<0.001
Dentine (Per-Protocol population)	0.42	0.33 to 0.53	<0.001

† Ratio of means larger than 1 indicates more erosion with Sensodyne ProNamel® 1426 ppm Fluoride + 5% potassium nitrate than with placebo.

In a similar way to enamel, it was noted to be an imbalance between the groups (as defined by sequence of administration of products) with those subjects administered fluoride first having higher levels of surface loss in both periods than those administered placebo first. Table 4.12 indicated that surface loss was greater during the placebo period than during the fluoride period for enamel.

Table 4.12: Summary of dentine surface loss (µm) from baseline to Day 21 (Intention to Treat Population)

Group	variable	N	Mean	SD	max	min
Pronamel/Placebo	Pronamel	19	19.29	18.39	79.49	2.68
	Placebo	19	40.75	30.65	135.77	2.58
Placebo/Pronamel	Pronamel	18	12.55	18.09	74.49	2.28
	Placebo	18	26.48	26.47	109.47	3.49
Total	Pronamel	37	16.01	18.31	79.49	2.28
	Placebo	37	33.81	29.20	135.77	2.58

4.4 Discussion

This section is divided into two parts. In the first section, the study design and the subject selection will be discussed. In the second part of the chapter the results of this study will be discussed and compared with other studies. Moreover, the clinical relevance of this study will be evaluated.

4.4.1 In situ study

In situ studies are being widely carried out in dental research as they simulate the natural oral processes better than animal or *in vitro* studies without being as time consuming or costly as *in vivo* studies. Furthermore; in situ studies allow for better control of the study subjects and better compliance than *in vivo* studies as the latter last longer (Zero, 1995). Intra-oral models involve less subjects and use *in vitro* measurement techniques which are very sensitive resulting in observation of the desired effect in much less time than when conducting an *in vivo* study. However, an *in situ* study can only be considered as an intermediate step between *in vitro* or animal and *in vivo* studies and should not be overestimated against *in vivo* studies and results are carefully extrapolated (Manning and Edgar, 1992).

4.4.2 Study design

Various *in situ* designs have been implemented depending on the specific parameters which are to be evaluated. Cross-over designs have been commonly used in intra-oral models as they have the advantage of using the same subject as its own control and therefore facilitating the process of subject selection and decreasing the number of volunteers required. However, these study designs last longer and consequently compliance might be compromised as the same subject participates for longer in the study. In addition, a carry-over effect from the treatment of the first leg might be a disadvantage of a cross-over study. A one-week wash-out period was included in the design of this study.

4.4.3 Subject selection

In every study the sample used should be representative of the population in which the results are intended to be applied to and therefore a randomised selection of the sample is advocated. In the majority of the *in situ* studies, either when investigating caries

processes and fluoride treatments or when assessing erosion or abrasion, the panellists are adults. The same age criteria were followed in the current study, as adults are more likely to comply with clinical protocols and generally have greater availability for appointments. Moreover, informed consent issues do not pose a problem in adults as opposed to children. It can be advocated anyway, that as the caries rate and the response to fluoride treatments are similar in both adults and children, the results of the study can also be applied to children (Zero, 1995).

Thirty seven volunteers were either dental students or dental nurses, which might be considered as an experimental bias (Zero, 1995). However, the results were based on objective observation and measurements by profilometry and not on subjective reports of symptoms by the volunteers. Therefore, the value of this type of bias seems insignificant.

All the volunteers were screened according to the recommendations by Curzon and Hefferren (2001) for intra-oral cariogenicity and erosion studies. The inclusion and exclusion criteria allow for standardisation of the different parameters that influence the caries process and erosion in order to enable us to expect a result in a short time and with a small sample size. Therefore, all the subjects had sound dentitions, with at least 18 teeth with no signs of periodontal disease (Löe plaque index=0-1, Löe gingival index=0, with BPE=0) (Löe, 1967) or erosion. Although the ultimate goal of these in situ models is people with dental disease, the panellist compliance with the prescribed regimen is of paramount importance and therefore, poor oral hygiene and dental neglect could be presumed as an indicator of compliance. Furthermore, edentulous subjects or dentitions with many missing teeth were found to have different micro flora than fully dentate subjects. On the other hand, periodontal disease will alter the microbiological consistency of the plaque whereas the presence of gingival recession areas seems to alter the fluoride clearance in the mouth (Zero, 1995). In addition, an intra-oral appliance would have deteriorated the periodontal problems. The detection of erosive defects during the screening test on the other hand, might reveal the presence of certain risk factors for erosion such as gastro-oesophageal reflux disease or eating disorders which would have influenced the results of this study.

The subjects had a normal salivary flow rate in order to ensure a normal response to the cariogenic and erosive challenge. Furthermore, in order to eliminate the effect of the background diet of the subjects on the degree of demineralisation of the enamel slabs,

the volunteers were instructed to leave the appliance out of their mouths when eating or drinking

The sample size of this study was achieved using a power calculation of 90% for enamel and 80% for dentine. However, the demanding laboratory procedures were time consuming and needed long preparation periods. All of this was achieved by the principal investigator.

4.4.4 Experimental appliance

A palatal removable denture with Adams clasps was used in this study. Although the appliance we used was developed by us and was not has not been used before, similar appliance designs have been used in previous erosive models (West *et al.*, 1998; Hara *et al.*, 2009; Magalhães *et al.*, 2008b). The advantage of the palatal appliance is that the potential for plaque retention is less. In addition, the appliance used in our study could be supported by observational studies (Sangnes and Gjermo, 1976) in which one of the more prevalent locations of abrasive dental lesions was the palatal surfaces of the upper teeth.

The design of the appliance used with the wires that were fitted had not been used in any other study. It was noticed that this design provided a stable fitting of the slabs and did not allow tongue action on the slabs. In order for this design to be applied, the slabs had to be relatively small (3 mm x 2 mm), which did not affect the study in any way. The only difficulty encountered was when the varnish was applied, which was very critical and care was taken to leave a clean exposed window. In spite of this, with the application of sticky wax on the top of the moulds no incidence of varnish loss was observed.

The effect of the appliance on the salivary parameters of each subject was assessed. Saliva from the subjects was collected on the screening day and one week after the subjects wore the appliance in order to investigate whether the appliance influenced the experimental parameters and therefore whether the *in situ* appliance created conditions different to the in vivo situation. No difference in salivary flow rate, either stimulated or unstimulated, was detected. These results were similar to those of Jaeggi and Lussi (1999) but different to those by Lussi *et al.*, (2004b), where the stimulated salivary flow rate of the subjects when they wore their appliance was statistically significantly less than when they did not wear the appliance. In the latter study, however, it was not clear

how much time was allowed for each volunteer to adapt to appliance wearing before saliva was collected.

4.4.5 Experimental Procedure

A three week period for each study arm was chosen in order to have sufficient time to produce changes in the surface profiles of the enamel and dentine slabs.

The compliance of the subjects was checked using the diary cards. No deviation from the instructed protocol could be detected through these compliance indicators. Despite this, one volunteer lost their appliances during the study.

4.4.6 Statistical methods

The two co-primary efficacy variables were

1. Enamel surface loss from baseline to Day 21.
2. Dentine surface loss from baseline to Day 21

An average was calculated for both variables using the measurements obtained from the two slabs at each assessment.

A preliminary examination of the data suggested that the variability of surface loss was dependent upon the size of the measurement i.e. larger measurements were associated with larger variability. Thus, the data was logged (using natural logarithm) for analysis. The primary efficacy variables were modelled using an ANOVA model appropriate for an AB/BA crossover design, incorporating investigational product, period, and subject, all as fixed effects. The differences between the products were estimated with 95% confidence intervals. The point estimate and confidence intervals were anti-logged to provide an estimate of the ratio of geometric means with a corresponding 95% confidence interval.

In order to assess the relationship between salivary flow rate and surface loss a regression model was fitted to outcomes obtained in the active phase of the study, incorporating sequence and salivary flow rate (both stimulated and unstimulated). The significance of this association was assessed using the p-value of the coefficient for salivary flow.

4.4.7 Discussion of results

The results of this study showed that sodium fluoride significantly decreased the amount of tooth surface loss compared to the placebo. This was evident in our previous *in vitro* model when we used an erosive challenge without combination with an abrasive component. This has been supported in the recent literature. Hara *et al.* (2009) compared dentifrices containing similar sources/concentrations of fluoride on the remineralisation of eroded enamel in situ. They recruited fifty-three subjects in a double-blind crossover study with three randomly assigned dentifrice treatments: placebo (0 ppm NaF, PD); reference (1,450 ppm NaF, RD) and test (1,450 ppm NaF + 5% KNO_3, TD). The researchers suggested that fluoride dentifrices provided erosion resistance for bovine enamel. This study compared the effect of fluoride toothpaste on the surface loss of bovine enamel using a short exposure to an erosive challenge and a single application of fluoride with a short in situ scenario. This protocol differed from our model, however it showed similar outcomes. We believe that in our model that we used long *in situ* scenario; the presence of high standard deviation and the variable results per subjects were expected. This is what was observed in the actual results. On the other hand, Rios *et al.* (2008) proposed the use of fluoride for the prevention of enamel wear; therefore they conducted an in situ, *ex vivo* study to assess the efficacy of a highly concentrated fluoride dentifrice on bovine enamel subjected to erosion and abrasion. The authors conducted a double-blind, crossover in situ study consisting of three phases (seven days each). In each phase, the authors tested one of the dentifrices (5,000 ppm F; 1,100 ppm F; no F). They performed erosive challenges with the use of a cola drink (60 s, four times per day) and abrasive challenges via toothbrushing (30 seconds, four times per day). The authors determined the enamel loss via profilometry and tested the data by using a two-way analysis of variance ($p \leq 0.05$). For the erosion plus abrasion condition, the study results showed that enamel wear was significantly higher than that with erosion alone. However, the findings showed no significant differences between the dentifrices regarding enamel wear. The authors concluded that the highly concentrated fluoride dentifrice did not have a protective effect on enamel against erosion and erosion plus toothbrushing abrasion. This was a different scenario with a different outcome to our results. It is perhaps possible that the short period of the *in situ* scenario was not enough to demonstrate the difference between the toothpastes used.

Magalhães *et al.*, (2008b) from the same group of Rios *et al.*, (2008) in an in situ/ex vivo study, assessed the effect of different concentrations of fluoride in dentifrices on dentine subjected to erosion or to erosion plus abrasion. The investigators concluded that the presence of fluoride concentrations around 1100 ppm F in dentifrices was important to reduce dentine wear by erosion and erosion + abrasion, but the protective effect did not increase with fluoride concentration. As in the study of Hara *et al.*, (2009), this model used a different protocol than our model. However, the results indicated a similar scenario.

Ganss *et al.*, (2007a) studied the stability of CaF_2-like precipitates on enamel and dentine under neutral or acidic conditions and compared *in vitro* and *in situ* results. They used human enamel and dentine specimens. The finding of this study, as described in the introduction of this chapter, could explain the significant difference between the placebo and fluoride groups in our study. It is possible that the stability of KOH-soluble fluoride in the in situ scenario caused the difference between those two groups.

Hunter *et al.*, (2003) investigated *in vitro* the effect of different fluoride preparations on erosion attributed to citric acid and citric acid-based soft drinks. Fluoride applied to enamel either in acidic solutions or as a pre-treatment reduced enamel erosion; however, the actual clinical benefit appeared to be low. On the other hand, Young *et al.*, (2006) compared the effect of toothpastes containing SnF_2 or NaF on enamel dissolution using an *in vivo* model. The researcher concluded that the SnF_2 toothpaste markedly reduced the dissolution of teeth *in vivo* (etch II < etch I), whereas the NaF toothpaste provided no protection (etch II > etch I). This controversy in the effect of NaF was the reason for this research project to be conducted and a series of the *in vitro* and an *in situ* studies that followed to investigate this scientific question.

4.5 Conclusions

Less than half as much surface erosion occurred for both enamel and dentine when Sensodyne ProNamel® 1426ppm Fluoride + 5% potassium nitrate was used compared to the placebo. This rejects the main null hypothesis of the study. In addition, this corroborates the results of our previous *in vitro* work (chapter 3).

5 SUMMARY

The outcomes of this research project can be summarised as follows:

1. Using a cycling protocol that mimics the oral environment has proven very difficult to apply and required continuous work for a long period of time. However, our cycling methodology gave consistent results and this was successfully applied in our studies.
2. Surface loss of enamel was less than dentine across all of the *in vitro* and *in situ* studies. This was very similar to most of the reported findings of the other researchers.
3. Erosion was reduced by the increase of fluoride concentration in toothpaste. As far as we are aware, this is the first time that a dose response of fluoride for the reduction of erosion has been reported in a longitudinal erosion model.
4. Abrasion (toothbrushing) reduced the beneficial effect of fluoride toothpaste. This has important clinical implications. It indicates that a combination of multiple preventative agents is advisable for high-risk dental erosion patients.
5. The effect of the reduction of RDA levels in toothpastes did not have an impact on the reduction of the amount of tooth surface loss, especially for dentine. Possibly the force of toothbrushing had an impact on the amount of tooth surface loss. However, this theory needs further investigation using our model.
6. The amount of tooth surface loss of deciduous enamel was more than permanent enamel; however they had a similar trend. This might be due to the difference in the structure of deciduous and permanent enamel.
7. The preventative effect of fluoride on reduction of tooth surface loss was very clear when compared to placebo in our in situ scenario.

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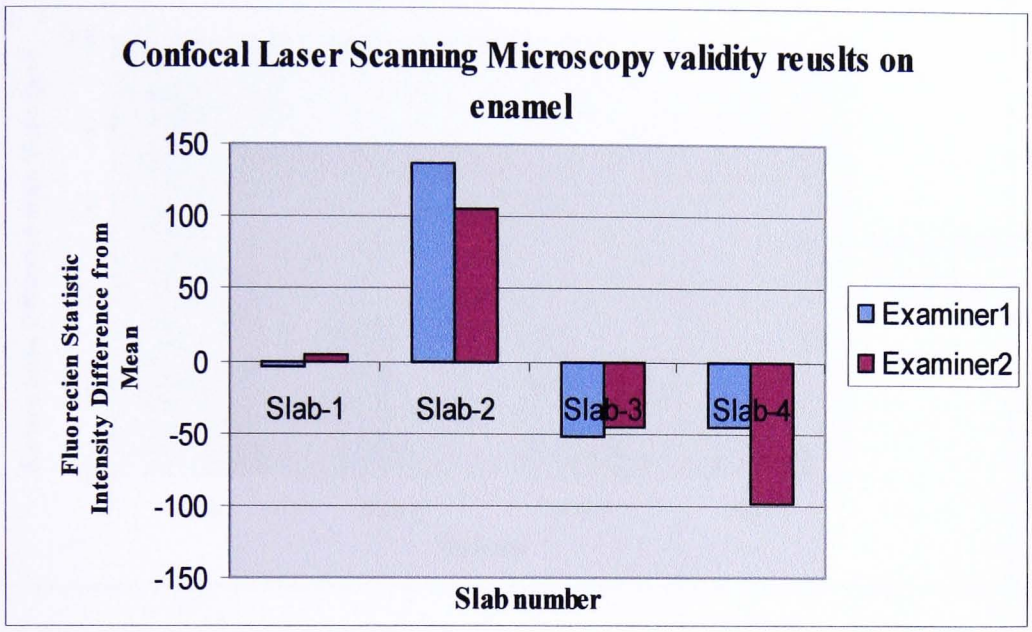
7 APPENDICES

Appendix 1



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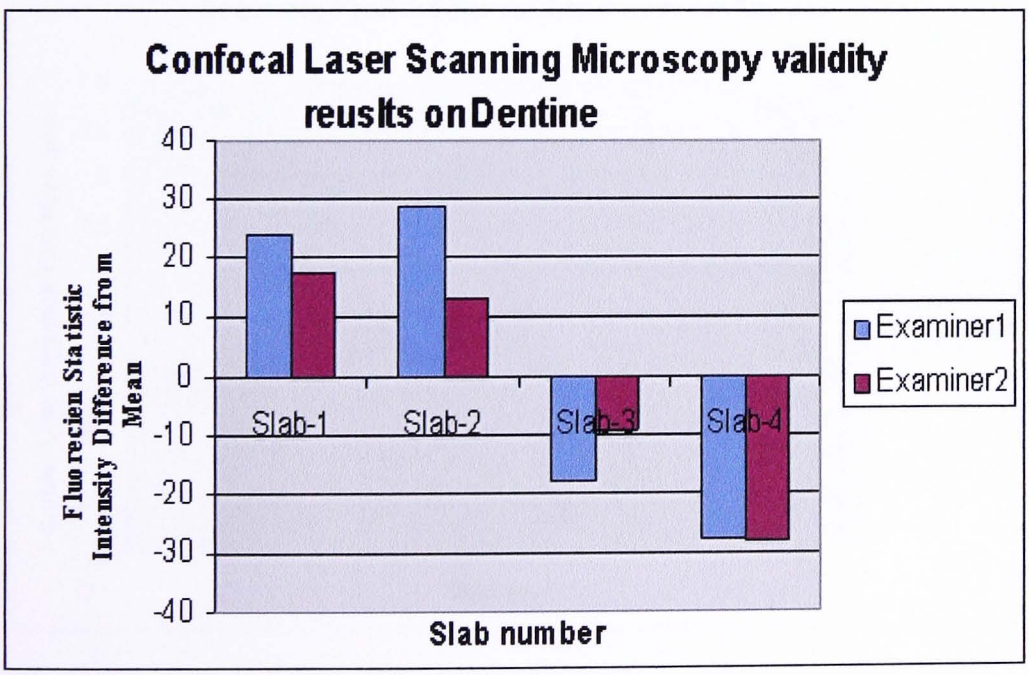

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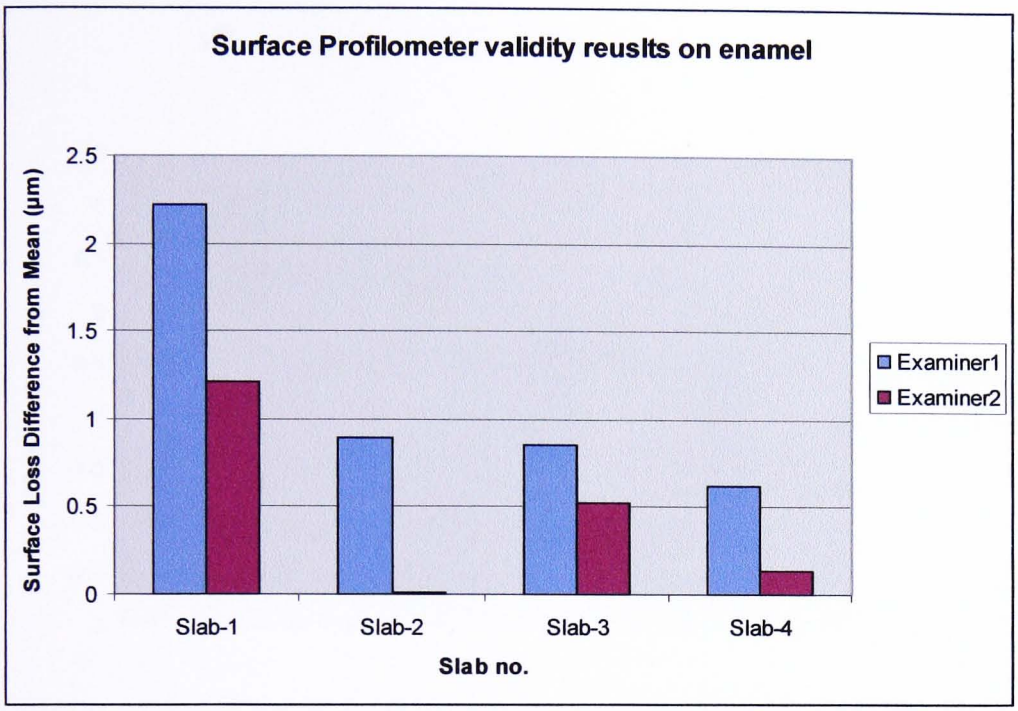

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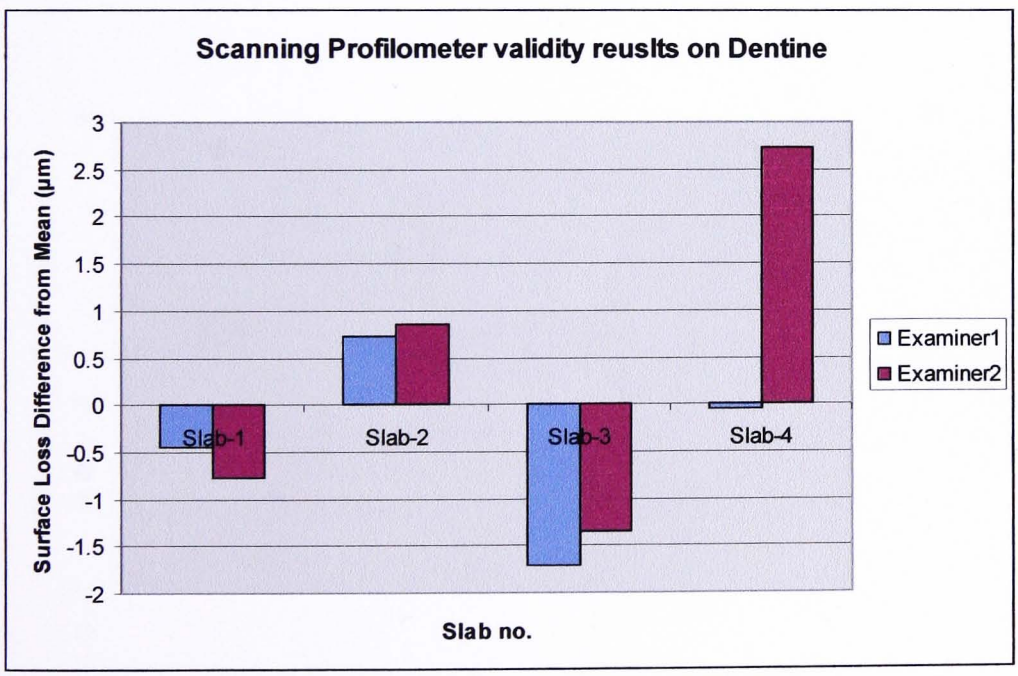

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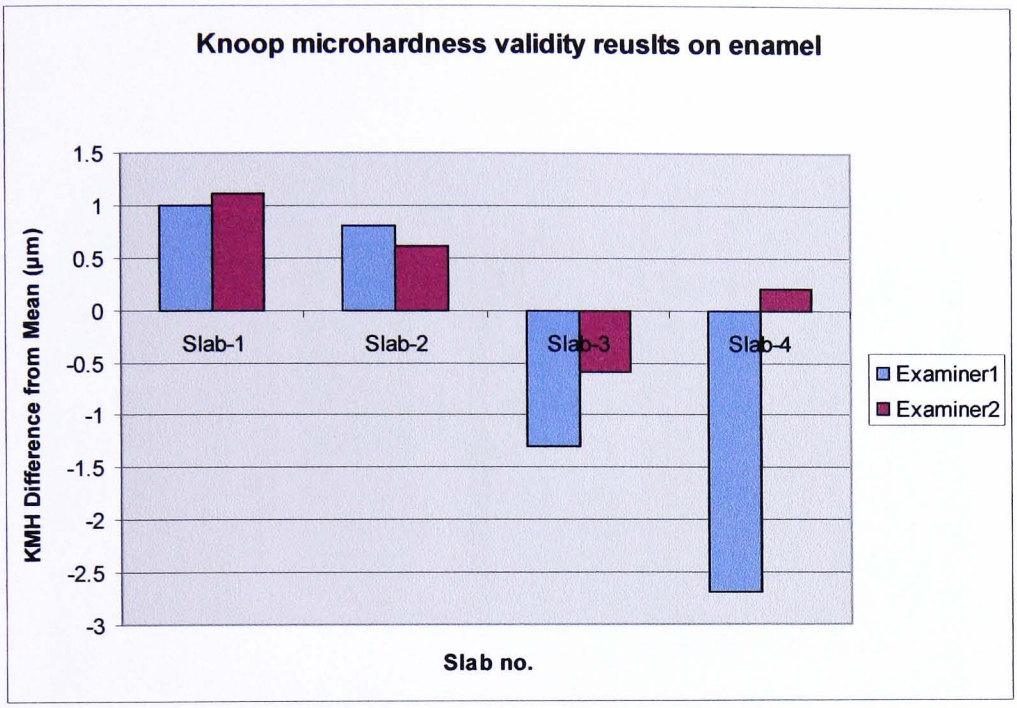

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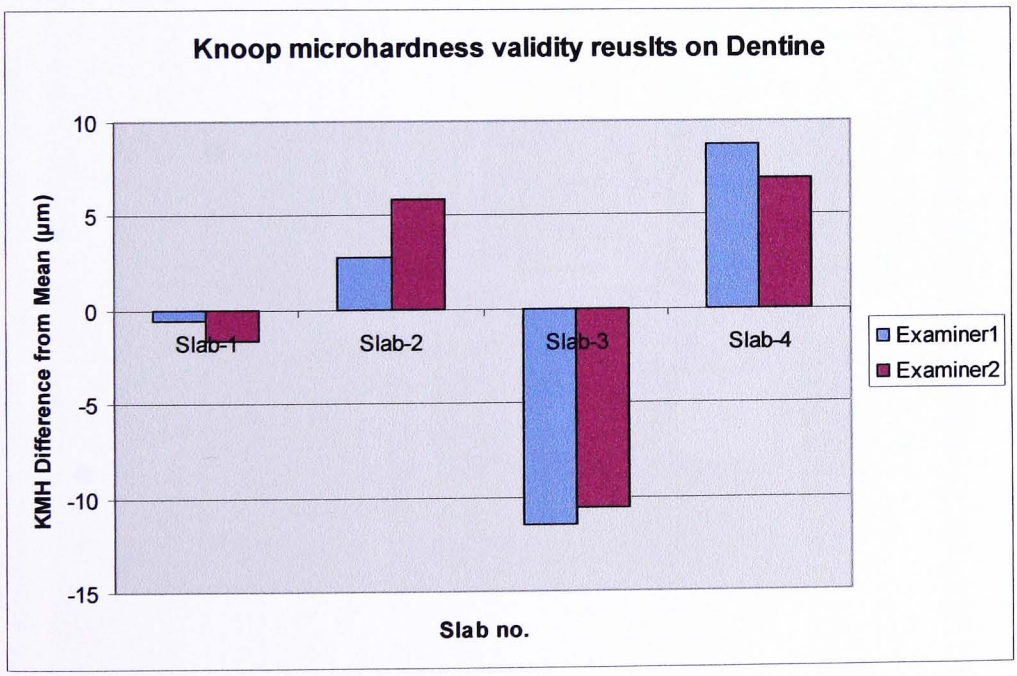

Appendix 7: Poster presented at IADR 2006 Congress, Brisbane, Australia.

Formation of erosive lesions on enamel and dentine in vitro

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The Leeds Teaching Hospitals NHS

INTRODUCTION

Modern diets contain multiple sources of potential acid exposure which depending on the frequency and manner of consumption increase the risk of dental erosion (Moazzez et al, 2000). In developing experimental models to investigate the effects of acid erosion on oral hard tissues it is important to try mimicking the in vivo scenario as closely as possible. In vitro studies on dental erosion have tried to create erosive lesions on enamel and/or dentine using different methods. In most of these in vitro studies erosive lesions are produced by simply immersing a tooth into soft drinks or orange juice for a prolonged period of time (Hunter et al., 2000, Lussi et al., 1995, Eisenburger and Addy, 2002). However, Amaechi et al. (1999) used a modified technique of dipping in orange juice (20 mL/specimen) at regular intervals six times per day for 5 minutes on each occasion for a period of 24 days, giving a total of 30 min daily. Using a modified procedure of the latter technique our study attempted to mimic typical daily acid exposure from different sources of dietary fruit acid in order to investigate enamel and dentine surface loss.

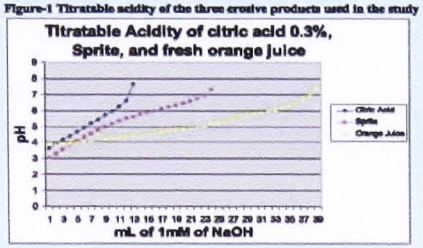
AIM

To evaluate human enamel and dentine surface loss from three different citric acid sources using an in vitro demin/ remin cycling technique.

MATERIALS AND METHODS

In this study enamel and dentine surface loss from three different citric acid sources [citric acid (0.3% pH 3.6), pure orange juice (pH 3.85), or Sprite (pH 3.05)] was evaluated using an in vitro demin/ remin cycling technique using surface profilometery. The titratable acidity of the three erosive products was measured by adding 1 ml of 1mM of NaOH to the erosive products. The pH was monitored using a pH electrode (VWR international Orion, Orion research, UK) (Figure-1).

Figure-1 Titratable acidity of the three erosive products used in the study



Three groups of eight enamel slabs and seven dentine slabs each were allocated randomly. The slabs were cut and mounted into resin blocks, ground and checked for surface flatness using a scanning profilometer (Scaniron Proscan 2000) Each slabs' surface was covered with nail varnish except for a small window (1x2 mm).
Each group was immersed under static conditions for 2 minutes, five times daily in fresh 200 ml aliquots of either citric acid 0.3% (pH=3.6), fresh orange juice from concentrate (pH=3.85) (DBC Food Services Ltd., Herts, UK), or Sprite (pH=3.05) (Coca Cola enterprise Ltd., Uxbridge, UK) at room temperature. The total cycling period lasted for 7 days during which time the slabs were placed into artificial saliva at 37°C between erosive challenges and overnight. The total exposure time was 70 minutes.

Before and after dipping into the erosive solutions the slabs were rinsed with de-ionised water. A gap of 60 minutes was left between immersions during which the slabs were returned to artificial saliva.
After the cycling period, the slabs were analysed with the scanning profilometer to measure the amount of surface loss caused by the different erosive products.
Confidence intervals were used to compare the erosive products. Related sample t-tests were used to calculate the confidence intervals since all the slabs came from the same origin (tooth).

RESULTS

1.Enamel

All the erosive products were observed to generate significant hard tissue surface loss. Surface loss on enamel caused by citric acid was 5.22±1.68 µm, by Sprite was 3.49±1.16 µm and by orange juice was 2.87±1.58 µm (Figure-2). However, the erosive lesions caused by citric acid were significantly different (CI 95%) from the erosive lesions caused by orange juice or Sprite (Table-1).

Figure-2 Mean ±SD changes of surface loss from baseline caused by the three erosive products [citric acid 0.3% (pH=3.6), pure orange juice from concentrate (pH=3.85), or Sprite (pH=3.05)]

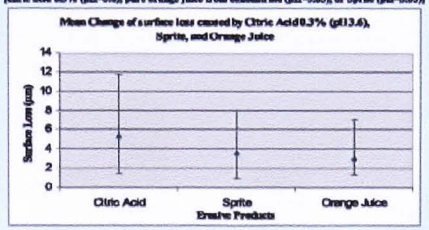


Table-1 Comparison of the mean change in surface loss (enamel wear) caused by citric acid 0.3% (pH3.6), orange juice and Sprite on creation of erosive lesion on dental enamel.

			n	Mean Diff	SD	95% Confidence Interval of the Difference	
						Lower	Upper
Citric Acid	VS	Sprite	8	1.73	1.11	0.80	2.66*
Citric Acid	VS	Orange Juice	8	2.35	1.58	1.03	3.67*
Sprite	VS	Orange Juice	8	0.62	1.22	-0.40	1.64

*Significant difference.

2. Dentine

As for enamel, there were measurable erosive lesions created by using all of the erosive products. Surface loss on dentine by citric acid, Sprite, and orange juice were 7.00±2.17, 7.45±2.78 and 7.64±1.91 µm respectively (Figure-3).
However, there were no significant differences between erosive products when comparing the confidence intervals of the paired t-test (Table-2).

Figure-3 Mean changes in surface loss (enamel wear) caused by citric acid 0.3% (pH3.6), orange juice and sprite on creation of erosive lesions on dentine. Error bars represent the confidence intervals.

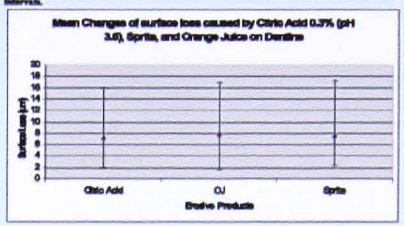


Table-2 Comparison of the mean changes in surface loss (enamel wear) caused by citric acid 0.3% (pH3.6), orange juice and sprite on creation of erosive lesions on dentine.

			n	Mean Diff	SD	95% Confidence Interval of the Difference	
						Lower	Upper
Citric Acid	VS	Sprite	7	-0.45	3.33	-3.23	2.34*
Citric Acid	VS	Orange Juice	7	-0.63	3.31	-3.40	2.13*
Sprite	VS	Orange Juice	7	-0.19	1.55	-1.48	1.11*

* Significant difference

CONCLUSION

- All three agents produced measurable erosive lesions in dentine and enamel.
- In this study differences in titratable acidity did not result in statistically significant differences in surface loss measured by profilometry.
- This *in vitro* cycling model could be used to investigate the role of preventative agents in inhibiting surface loss progression from erosion.

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Produced by AZ Abdullah and Medical & Dental Illustration

Appendix 8 Poster presented at PEF IADR 2007 Congress, Dublin, Ireland.

Effect of temperature on enamel surface loss in vitro

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GlaxoSmithKline

Objective

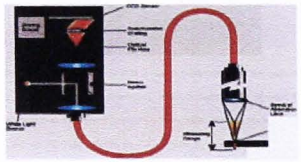
To evaluate the effect of varying the temperature of a citric acid challenge on human enamel surface loss *in vitro* using a brushing based pH cycling model.

Introduction

Tooth wear is recognised as a major problem in both children and adults[1]. It is a multi-factorial problem generally initiated following dissolution of mineralised tooth structure after contact with acids that are introduced into the oral cavity from Intrinsic (e.g., gastroesophageal reflux, vomiting) or extrinsic sources (e.g., acidic beverages, citrus fruits)[2] Mechanical factors, such as abrasion from toothbrushing or attrition acting on the demineralised surface, can lead to tissue loss. It is reasonable to expect that factors such as pH, duration of exposure and temperature will all impact the extent of surface removed[3,4]. The aim of these experiments were to investigate the temperature of an erosive challenge effects enamel surface loss in the presence of brushing with a fluoridated toothpaste.

Methods

Two similarly designed, randomised, blinded experiments were conducted sequentially. Each experiment involved three groups of eight enamel slabs cut and mounted into resin blocks, ground and checked for surface flatness using a scanning profilometer (Scantron Proscan 2000).

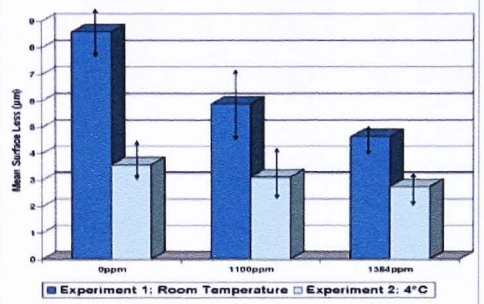


Each group was immersed under static conditions for 2 minutes, five times daily in fresh 200 ml aliquots of citric acid 0.3% (pH=3.6) at either room temperature (experiment 1) or 4° C (experiment 2).

Slabs were randomly assigned to undergo manual brushing twice daily, once before and the other after citric acid cycling, with either a 0ppm, 1100ppm or 1384ppm fluoride toothpaste. The 0ppm and 1384ppm toothpaste were abrasivity matched. Cycling periods in both experiments lasted for 7 days during which time slabs were incubated overnight and between erosive challenges in artificial saliva at 37° C. After cycling the slabs were analysed with the scanning profilometer to measure the amount of surface loss.

Results

Figure 1: Comparison of mean enamel surface loss (µm) after 7 days cycling with citric acid (pH3.6) at room temperature and 4° C



Experiment 1:

- Mean enamel surface loss (SD) in experiment 1 was 8.59µm (0.96), 5.87µm (1.07) and 4.63µm (0.70) for 0ppm, 1100ppm and 1384ppm fluoride toothpastes respectively.
- Differences in the extent of enamel surface loss observed between both fluoride containing and fluoride free toothpastes were statistically significant ($p<0.05$).
- No other statistically significant differences were observed

Experiment 2:

- Mean surface loss (SD) in experiment 2 was 3.57µm (0.84), 3.12 µm (0.84) and 2.75 µm (0.45) for 0ppm, 1100ppm and 1384ppm fluoride toothpastes respectively.
- Differences in the extent of enamel surface observed between 1384ppm toothpaste and fluoride free toothpaste was statistically significant ($p<0.05$).
- No other statistically significant differences were observed

Conclusions

In this erosion brushing-based cycling tooth wear in vitro model:

- **Enamel surface loss is temperature dependant of the acid challenge**
- **Brushing with fluoride toothpaste significantly reduces the extent of enamel surface loss.**

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Appendix 9: Confocal Laser Scanning Microscopy reproducibility results (fluorescein intensity) on dental enamel using dipping in 0.3% citric acid (pH 3.6) for one week.

		Integration into Enamel Surface					Mean
		1 um	5um	10um	15um	20um	
Slab 1	ROI 1:*	45.18	47.78	50.62	53.32	55.64	50.51
	ROI 2:†	15.295	15.53	15.75	16.02	16.40	15.80
Slab 2	ROI 1:*	90.17	95.50	104.68	112.03	115.94	103.66
	ROI 2:†	24.615	24.89	25.36	25.74	25.83	25.28
Slab 3	ROI 1:*	253.56	253.71	253.85	253.93	253.99	253.81
	ROI 2:†	33.93	35.14	37.20	38.71	39.48	36.89
Slab 4	ROI 1:*	135.03	142.93	151.16	163.09	171.26	152.69
	ROI 2:†	38.73	38.94	39.04	38.95	38.43	38.82
Slab 5	ROI 1:*	144.76	155.88	171.33	186.96	201.55	172.10
	ROI 2:†	15.075	16.40	18.06	19.32	20.10	17.79
Slab 6	ROI 1:*	219.65	222.12	223.33	222.98	220.85	221.79
	ROI 2:†	18.72	19.30	19.00	17.67	15.74	18.09
Slab 7	ROI 1:*	95.83	102.86	111.77	119.47	123.88	110.76
	ROI 2:†	25.17	25.26	25.33	25.09	24.67	25.10

*** Statistical Intensity of Fluorescein in the Eroded Area**
† Statistical Intensity of Fluorescein in the Intact Area

Appendix 10: Confocal Laser Scanning Microscopy reproducibility results (fluorescein intensity) on dentine using dipping in 0.3% citric acid (pH 3.6) for 3 days.

		Integration into Enamel Surface					Mean
		1 um	5um	10um	15um	20um	
Slab 1	ROI 1:*	67.26	68.86	70.65	74.38	77.23	71.68
	ROI 2:†	10.31	10.06	10.69	10.43	10.87	10.47
Slab 2	ROI 1:*	110.66	106.10	99.51	93.14	83.52	98.59
	ROI 2:†	0.02	0.02	0.02	0.01	0.01	0.02
Slab 3	ROI 1:*	143.93	146.44	150.55	154.45	158.19	150.71
	ROI 2:†	0.00	0.00	0.00	0.00	0.01	0.00
Slab 4	ROI 1:*	57.99	55.98	53.71	48.92	45.33	52.39
	ROI 2:†	0.10	0.09	0.08	0.07	0.05	0.08
Slab 5	ROI 1:*	121.77	119.21	114.99	109.68	106.15	114.36
	ROI 2:†	0.48	0.49	0.43	0.46	0.39	0.45
Slab 6	ROI 1:*	98.55	98.26	95.63	91.32	85.86	93.92
	ROI 2:†	0.03	0.03	0.03	0.03	0.03	0.03
Slab 7	ROI 1:*	68.69	69.00	67.71	65.04	61.56	66.40
	ROI 2:†	0.15	0.17	0.17	0.18	0.19	0.17

*** Statistical Intensity of Fluorescein in the Eroded Area**
† Statistical Intensity of Fluorescein in the Intact Area

Appendix 11: Knoop microhardness reproducibility results (μm) on dental enamel using dipping in 0.3% citric acid (pH 3.6) for one week.

	Indent no.	Baseline				Treatment			
		1st indent	2nd indent	3rd indent	Mean	1st indent	2nd indent	3rd indent	Mean
Slab 1	1	67.3	67.5	67.7	67.5	162.7	167.4	164.6	164.9
	2	61.1	60.9	61.6	61.2	159.9	167.0	154.0	160.3
	3	64.7	65.4	65.5	65.2	147.6	147.0	148.0	147.5
	4	66.1	66.3	68.0	66.8	148.6	152.9	150.0	150.5
	5	61.4	60.4	61.1	61.0	149.3	156.2	156.2	153.9
	Mean	64.1	64.1	64.8	64.3	153.6	158.1	154.6	155.4
Slab 2	1	63.7	63.7	64.2	63.9	109.8	109.4	111.0	110.1
	2	70.6	68.9	69.1	69.5	107.5	107.0	107.0	107.2
	3	62.6	61.1	64.0	62.6	98.3	98.5	98.3	98.4
	4	60.7	62.3	61.1	61.4	101.4	101.6	101.6	101.5
	5	57.6	58.1	58.1	57.9	108.4	109.1	108.7	108.7
	Mean	63.0	62.8	63.3	63.1	105.1	105.1	105.3	105.2
slab 3	1	68.7	69.4	69.1	69.1	103.0	106.1	106.0	105.0
	2	66.1	66.1	66.6	66.3	114.8	118.3	115.9	116.3
	3	64.0	66.1	66.6	65.6	119.5	119.7	118.5	119.2
	4	64.2	64.2	63.0	63.8	120.6	120.5	120.3	120.5
	5	64.0	63.7	64.4	64.0	121.1	121.8	121.8	121.6
	Mean	65.4	65.9	65.9	65.7	115.8	117.3	116.5	116.5
Slab 4	1	69.9	68.9	68.9	69.2	121.6	121.7	121.6	121.6
	2	64.9	65.4	64.7	65.0	131.2	133.6	131.7	132.2
	3	60.9	62.1	62.0	61.7	137.8	138.5	132.9	136.4
	4	69.1	68.7	68.4	68.7	133.3	133.5	133.1	133.3
	5	65.4	66.8	66.3	66.2	134.5	134.3	134.3	134.4
	Mean	66.0	66.4	66.1	66.2	131.7	132.3	130.7	131.6
Slab 5	1	68.0	67.7	69.8	68.5	111.2	111.5	111.5	111.4
	2	67.7	68.2	66.1	67.3	111.9	111.9	111.9	111.9
	3	65.4	65.6	66.1	65.7	117.4	111.6	117.2	115.4
	4	64.4	63.5	64.4	64.1	116.9	116.8	116.8	116.8
	5	64.0	64.9	66.8	65.2	120.6	118.1	118.1	118.9
	Mean	65.9	66.0	66.6	66.2	115.6	114.0	115.1	114.9
Slab 6	1	67.3	67.7	67.0	67.3	114.8	114.8	114.8	114.8
	2	62.8	62.3	62.1	62.4	103.0	103.0	103.0	103.0
	3	63.0	63.7	63.0	63.2	88.2	88.2	88.2	88.2
	4	62.6	64.2	60.7	62.5	91.5	91.5	91.6	91.5
	5	64.2	64.4	64.4	64.3	103.7	103.7	103.7	103.7
	Mean	64.0	64.5	63.4	64.0	100.2	100.2	100.3	100.2
Slab 7	1	65.8	67.7	65.8	66.4	111.7	111.7	111.7	111.7
	2	63.7	64.7	64.2	64.2	99.5	99.7	100.2	99.8
	3	66.3	66.3	65.4	66.0	102.3	103.1	102.5	102.6
	4	63.7	63.5	63.5	63.6	100.9	101.2	100.8	101.0
	5	64.9	62.8	64.4	64.0	107.7	107.5	107.9	107.7
	Mean	64.9	65.0	64.7	64.8	104.4	104.6	104.6	104.6
Slab 8	1	62.8	63.0	62.3	62.7	127.2	128.4	128.4	128.0
	2	61.6	62.6	62.3	62.2	136.7	137.3	137.8	137.3
	3	59.0	60.2	59.5	59.6	130.5	131.0	130.5	130.7
	4	64.0	64.9	64.7	64.5	111.5	111.5	111.5	111.5
	5	62.3	61.1	61.1	61.5	132.2	131.9	132.0	132.0
	Mean	61.9	62.4	62.0	62.1	127.6	128.0	128.0	127.9

Appendix 12: Knoop microhardness reproducibility results (µm) on dentine using dipping in 0.3% citric acid (pH 3.6) for 3 days.

	Indent no.	Baseline				Treatment			
		1st indent	2nd indent	3rd indent	Mean	1st indent	2nd indent	3rd indent	Mean
Slab 1	1	79.3	81.8	81.1	80.7	110.3	107.9	108.2	108.8
	2	89.4	90.8	88.2	89.5	107.0	102.1	106.1	105.1
	3	88.1	86.2	90.8	88.4	118.4	118.8	122.1	119.8
	4	82.6	88.1	85.3	85.3	115.0	112.6	115.9	114.5
	5	78.0	78.0	79.8	78.6	100.7	97.6	103.0	100.4
	Mean	83.5	85.0	85.0	84.5	110.3	107.8	111.1	109.7
Slab 2	1	101.1	101.0	101.1	101.1	147.5	142.0	141.3	143.6
	2	107.5	106.3	106.3	106.7	120.9	129.3	119.5	123.2
	3	93.4	92.0	93.1	92.8	117.6	120.9	115.2	117.9
	4	95.5	96.7	95.5	95.9	123.9	130.0	124.9	126.3
	5	94.1	94.1	94.1	94.1	109.7	111.7	117.1	112.8
	Mean	98.3	98.0	98.0	98.1	123.9	126.8	123.6	124.8
slab 3	1	94.3	95.2	92.4	94.0	105.8	104.7	104.7	105.1
	2	86.8	85.6	84.7	85.7	103.2	107.2	105.6	105.3
	3	87.8	83.7	87.7	86.4	114.1	111.5	114.3	113.3
	4	81.1	83.5	84.0	82.9	117.1	116.6	115.9	116.5
	5	80.7	81.6	82.5	81.6	118.5	120.2	125.3	121.3
	Mean	86.1	85.9	86.3	86.1	109.7	111.7	117.1	112.8
Slab 4	1	80.0	83.3	78.3	80.5	123.0	119.9	123.2	122.0
	2	85.4	83.0	80.9	83.1	91.2	96.1	91.2	92.8
	3	81.6	81.1	81.1	81.3	96.9	96.9	96.9	96.9
	4	77.4	80.0	79.3	78.9	97.1	99.5	99.5	98.7
	5	79.3	80.2	78.8	79.4	92.7	92.2	93.0	92.6
	Mean	80.7	81.5	79.7	80.6	100.2	100.9	100.8	100.6
Slab 5	1	99.5	99.0	97.8	98.8	114.3	113.6	115.5	114.5
	2	94.5	94.8	92.4	93.9	114.2	112.8	117.8	114.9
	3	96.0	99.5	99.0	98.2	120.1	124.4	124.2	122.9
	4	99.2	97.8	93.8	96.9	116.4	113.1	113.8	114.4
	5	80.7	80.4	80.6	80.6	156.6	159.1	160.2	158.6
	Mean	94.0	94.3	92.7	93.7	124.3	124.6	126.3	125.1
Slab 6	1	82.1	84.2	83.3	83.2	104.9	106.8	106.8	106.2
	2	78.3	82.1	77.8	79.4	96.0	94.1	94.5	94.9
	3	72.2	72.2	72.2	72.2	112.4	116.9	115.7	115.0
	4	88.7	88.9	89.1	88.9	112.4	112.4	112.4	112.4
	5	89.6	89.8	90.8	90.1	106.5	106.8	106.9	106.7
	Mean	82.2	83.4	82.6	82.8	106.4	107.4	107.3	107.0
Slab 7	1	78.5	80.2	83.3	80.7	105.8	108.2	108.4	107.5
	2	84.0	81.8	79.3	81.7	115.0	111.0	112.6	112.9
	3	83.0	87.7	90.8	87.2	101.1	104.2	103.5	102.9
	4	85.4	81.4	83.5	83.4	119.0	118.1	120.4	119.2
	5	78.3	83.5	81.6	81.1	125.8	124.9	123.9	124.9
	Mean	81.8	82.9	83.7	82.8	113.3	113.3	113.8	113.5

Appendix 13: Scanning electron microscopy reproducibility results (Ca:P ratios) on dental enamel using dipping in 0.3% citric acid (pH 3.6) for one week.

	Intact Enamel Surface						Eroded Enamel Surface					
	1st reading	2nd reading	3rd reading	4th reading	5th reading	Mean	1st reading	2nd reading	3rd reading	4th reading	5th reading	Mean
Slab-1	1.58	1.60	1.72	1.63	1.55	1.62	1.66	1.66	1.67	1.58	1.49	1.61
Slab-2	1.68	2.13	1.77	1.65	1.75	1.80	1.71	1.73	1.96	1.86	1.99	1.85
Slab-3	1.57	1.50	1.49	1.37	1.48	1.48	1.53	1.53	1.53	1.46	1.52	1.51
Slab-4	1.58	1.63	1.61	1.57	1.43	1.57	1.67	1.63	1.64	1.52	1.62	1.62
Slab-5	1.70	1.57	1.56	1.44	1.45	1.54	1.69	1.79	1.60	1.70	1.63	1.68
Slab-6	1.50	1.46	1.52	1.58	1.65	1.54	1.61	1.66	1.58	1.64	1.51	1.60
Slab-7	1.49	1.72	1.63	1.77	1.61	1.64	1.63	1.74	1.59	1.76	1.83	1.71

Appendix 14: Scanning electron microscopy reproducibility results (Ca:P ratios) on dentine using dipping in 0.3% citric acid (pH 3.6) for 3 days.

	Intact Dentine Surface						Eroded Dentine Surface					
	1st reading	2nd reading	3rd reading	4th reading	5th reading	Mean	1st reading	2nd reading	3rd reading	4th reading	5th reading	Mean
Slab-1	1.80	1.77	1.87	1.95	1.81	1.84	2.00	1.97	2.02	1.98	2.01	2.00
Slab-2	1.84	1.97	1.90	1.98	1.97	1.93	1.94	1.92	1.92	2.02	1.97	1.95
Slab-3	1.70	1.55	1.48	1.55	1.64	1.58	1.56	1.71	1.60	1.87	1.60	1.67
Slab-4	1.66	1.72	1.50	1.56	1.71	1.63	1.69	1.59	1.70	1.70	1.63	1.66
Slab-5	1.67	1.58	1.63	1.55	1.67	1.62	1.73	1.66	1.72	1.72	1.71	1.71
Slab-6	1.64	1.65	1.57	1.46	1.69	1.60	1.80	1.79	1.65	1.57	1.47	1.65
Slab-7	2.19	1.86	2.57	2.08	2.12	2.16	1.78	1.50	2.01	1.57	1.83	1.74

Appendix 15: Surface Profilometer reproducibility results (Surface Loss - µm) on dental enamel using dipping in 0.3% citric acid (pH 3.6) for one week.

Slab no.	1st reading	2nd reading	3rd reading	4th reading	5th reading	Mean
1	9.51	10.13	9.28	9.28	10.97	8.36
2	7.39	7.36	7.58	7.09	6.98	6.40
3	16.95	12.35	14.21	15.17	13.54	12.54
4	8.95	9.43	9.20	9.79	9.56	8.49
5	9.22	10.30	9.10	9.47	9.34	8.74
6	7.43	12.18	7.66	6.58	6.58	7.74
7	11.41	9.61	10.30	9.77	8.37	9.41
8	8.07	9.45	9.42	9.93	9.09	8.99

Appendix 16 Surface Profilometer reproducibility results (Surface Loss - µm) on dentine using dipping in 0.3% citric acid (pH 3.6) for 3 days.

Slab no.	1st reading	2nd reading	3rd reading	4th reading	5th reading	Mean
1	4.87	6.41	5.89	5.96	5.21	4.89
2	2.79	1.72	2.24	1.77	2.96	2.25
3	2.83	2.76	3.06	3.71	3.38	3.12
4	2.35	2.31	2.58	1.74	1.99	2.50
5	2.89	2.52	2.76	2.91	2.64	3.12
6	4.06	3.2	3.67	3.98	4.45	4.23
7	2.84	3.61	2.8	3.59	3.98	3.97

Appendix 17 Surface Profilometer reproducibility results (Surface Loss - µm) on dental enamel using dipping in 0.3% citric Acid (pH 3.6), Orange Juice, or Sprite for one week.

Slab no.	Citric Acid	Sprite	Orange Juice
1	7.71	4.66	2.55
2	6.77	4.79	5.77
3	6.76	3.50	4.19
4	3.33	2.52	2.39
5	4.86	2.80	1.08
6	3.53	1.63	0.99
7	5.02	4.68	2.63
8	3.78	3.31	3.36
Mean	5.22	3.49	2.87

Appendix 18 Surface Profilometer reproducibility results (Surface Loss - µm) on dental enamel using dipping in 0.3% citric Acid (pH 3.6), Orange Juice, or Sprite for one week.

Slab no.	Citric Acid	Sprite	Orange Juice
1	6.51	4.68	6.59
2	9.39	6.24	6.89
3	6.01	10.11	8.87
4	5.80	12.41	11.85
5	11.20	8.94	6.43
6	6.67	6.41	6.53
7	5.74	6.33	7.67
8	4.69	4.48	6.25
Mean	7.00	7.45	7.64

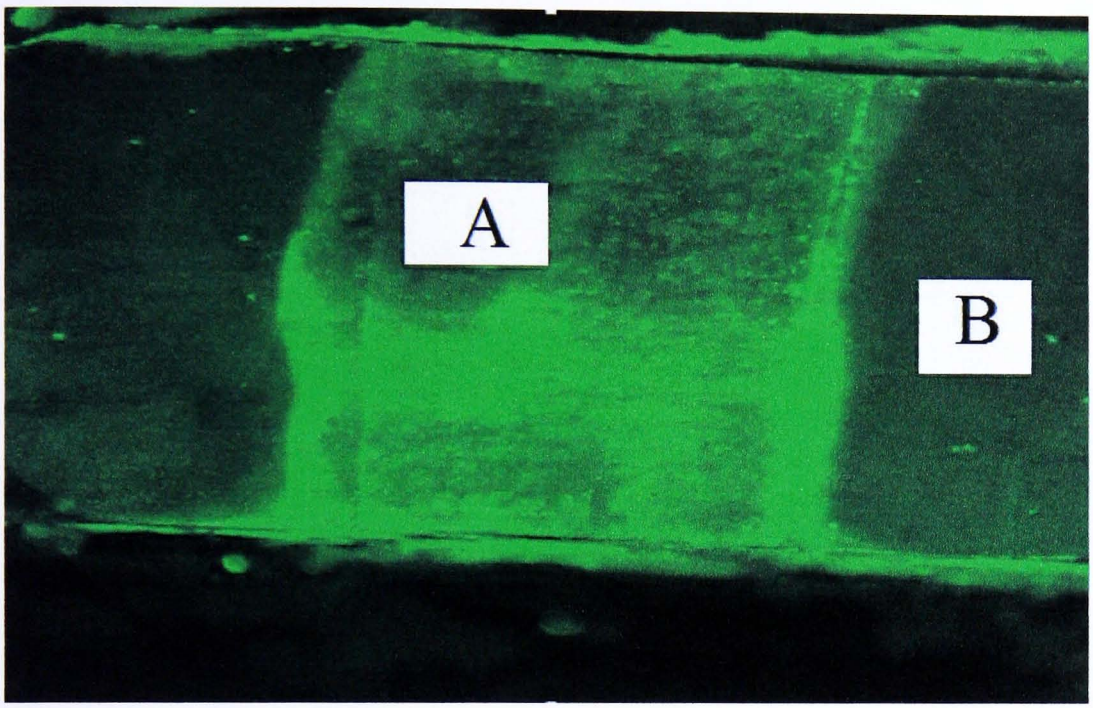
Appendix 19 Surface Profilometer reproducibility results (Surface Loss - µm) on dental enamel using dipping in 0.3% citric Acid (pH 3.6) one week in the presence of 3 different concentrations of fluoride toothpastes.

Slab no.	0 ppm F	1100 ppm F	1384 ppm F
1	12.46	7.02	4.80
2	9.05	5.84	4.34
3	8.17	5.85	3.91
4	6.03	5.23	2.26
5	8.39	5.96	9.01
6	7.73	7.19	4.07
7	8.32	4.00	5.39
Mean	8.59	5.87	4.83

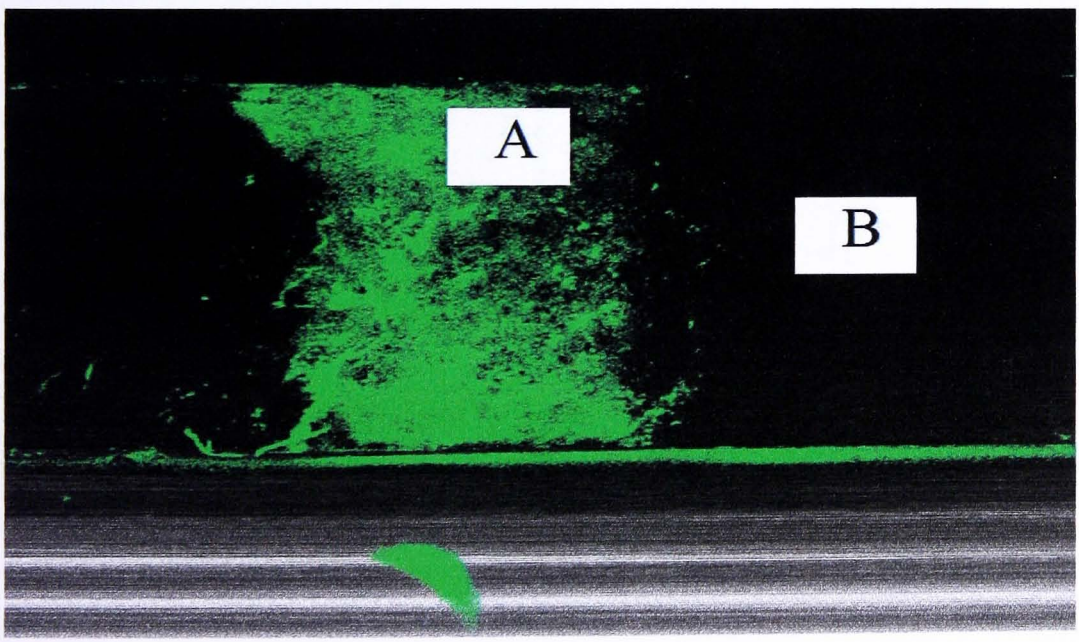
Appendix 20 Surface Profilometer reproducibility results (Surface Loss - µm) on dentine using dipping in 0.3% citric Acid (pH 3.6) one week in the presence of 3 different concentrations of fluoride toothpastes.

Slab no.	0 ppm F	1100 ppm F	1384 ppm F
1	5.14	5.25	3.87
2	6.01	4.22	4.57
3	5.74	2.70	3.33
4	4.69	3.60	4.18
5	6.67	4.39	4.96
6	5.80	2.40	5.73
7	9.39	4.69	4.85
Mean	6.21	3.89	4.50

Appendix 21: CLSM scan shows the integration of fluorescein into the enamel surface. (A) Eroded area. (b) Intact area.



Appendix 22: CLSM scan shows the integration of fluorocein into the dentine surface. (A) Eroded area. (b) Intact area.



Appendix 23: Poster presented at PEF IADR 2007 Congress, Dublin, Ireland.

Effect of different concentrations of fluoridated toothpaste on dental erosion

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INTRODUCTION

Dental erosion is defined as the irreversible loss of dental hard tissue by acids not involved in the carious process (Pindborg, 1970). The interest in dental erosion has increased dramatically over the last ten years. The effect of fluoride on the reduction of dental erosion was investigated in different studies (Attin et al., 2003; Lussi et al., 1993; and Amaechi et al., 1998). All investigators have reported that adding fluoride to potential erosive drinks (e.g., orange juice) has reduced the surface demineralisation of bovine enamel. Until recently, there are only a few studies that have investigated the role of fluoridated toothpaste on human enamel on the reduction or prevention of dental erosion.

AIM

To compare the effect of three different concentrations of fluoridated toothpastes on enamel erosion in vitro using surface profilometry.

MATERIALS AND METHODS

In a randomised, blinded experiment three groups of seven enamel slabs were cut and mounted into resin blocks, ground and checked for surface flatness using a scanning profilometer (Scantron Proscan 2000). Each slab's surface was covered with nail varnish except for a small window (1x2 mm). Each group was immersed under static conditions for 2 minutes, five times daily in fresh 200 ml aliquots of citric acid 0.3% (pH=3.6). In addition, the slabs were immersed in 3 different toothpastes (0ppm F, 1100ppm F, or 1450ppm F) twice daily, morning and evening, for 2 minutes each time. The total cycling period lasted 16 days. Slabs were incubated overnight and between erosive challenges in artificial saliva at 37oC. A sixty minute gap was left between daytime erosive challenges. Before and after dipping in the erosive solutions the slabs were rinsed with de-ionised water. The slabs were analysed with the scanning profilometer to measure the amount of surface loss at days 4, 8, 12, and 16.

RESULTS

Surface loss increased at all timepoints up to day 16 in all groups, with a maximum increase after day 12. Surface loss ± SD of enamel caused by citric acid combined with using non-fluoridated toothpaste at day 16 was 61.19±8.50 µm, 1100ppm F was 43.44±10.94 µm and 1450ppm F was 34.98±4.29 µm. Treatment differences were statistically significant (CI 95%) for all between product comparisons at day 16. no other statistical difference was observed between products for other timepoints.

Figure-1: Mean surface loss of enamel caused by cycling in citric acid 0.3% (pH=3.6) for 16 days and using 3 different fluoridated toothpastes (error bars are 95%CI).

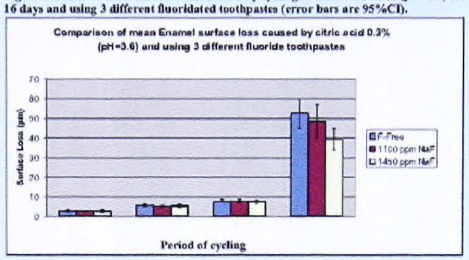

Table-1: Comparison between the effect of 0ppm F VS 1450ppm F toothpastes on enamel surface loss caused by cycling in citric acid 0.3% (pH=3.6) for 16 days

	t-test for Equality of Means				
	n	Mean Difference	SE Difference	95% Confidence Interval of the Difference	
				Lower	Upper
0ppm F VS 1450ppm F: 4 Days Treatment	7	0.10	0.26	-0.46	0.67
0ppm F VS 1450ppm F: 8 Days Treatment	7	0.68	0.56	-0.54	1.91
0ppm F VS 1450ppm F: 12 Days Treatment	7	0.96	0.62	-0.39	2.30
0ppm F VS 1450ppm F: 16 Days Treatment	7	26.21	3.60	18.36	34.05*

* Significant difference

Table-2: Comparison between the effect of 0ppm F vs 1100ppm F toothpastes on enamel surface loss caused by cycling in citric acid 0.3% (pH=3.6) for 16 days

	t-test for Equality of Means				
	n	Mean Difference	SE Difference	95% Confidence Interval of the Difference	
				Lower	Upper
0ppm F vs 1100ppm F: 4 Days Treatment	7	-0.13	0.50	-1.21	0.95
0ppm F vs 1100ppm F: 8 Days Treatment	7	-0.07	0.70	-1.61	1.46
0ppm F vs 1100ppm F: 12 Days Treatment	7	0.67	0.63	-0.71	2.05
0ppm F vs 1100ppm F: 16 Days Treatment	7	17.74	5.24	6.33	29.16*

* Significant difference

Table-3: Comparison between the effect of 1450ppm F VS 1100ppm F toothpastes on enamel surface loss caused by cycling in citric acid 0.3% (pH=3.6) for 16 days

	t-test for Equality of Means				
	n	Mean Difference	SE Difference	95% Confidence Interval of the Difference	
				Lower	Upper
1450ppm F VS 1100ppm F: 4 Days Treatment	7	-0.13	0.50	-1.21	0.95
1450ppm F VS 1100ppm F: 8 Days Treatment	7	-0.76	0.70	-2.28	0.77
1450ppm F VS 1100ppm F: 12 Days Treatment	7	-0.29	0.72	-1.85	1.27
1450ppm F VS 1100ppm F: 16 Days Treatment	7	-8.46	4.44	-18.14	-1.22*

* Significant difference

CONCLUSION

In this model fluoride toothpastes significantly reduced the progression of dental erosion.

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ACKNOWLEDGMENT

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Produced by AZ Abdullah and Medical & Dental Illustration

Appendix 24: Mean Change of Surface Loss from baseline caused by0.3% citric Acid (pH 3.6) while dipping in three different fluoride concentration toothpastes.

	Slab 1	Slab 2	Slab 3	Slab 4	Slab 5	Slab 6	Slab 7	Mean
0 ppm F								
Day-4	2.38	2.56	3.30	3.13	3.01	2.37	2.92	2.81
Day-8	4.82	5.30	5.56	7.78	5.62	4.55	6.16	5.68
Day-12	7.64	7.04	8.76	9.74	7.88	7.95	9.33	8.33
Day-16	56.64	51.63	60.84	64.81	51.54	73.17	69.68	61.19
1385 ppm F								
Day-4	3.03	3.22	3.15	1.63	3.00	2.35	2.56	2.71
Day-8	5.71	4.84	5.66	2.94	6.06	5.17	4.62	5.00
Day-12	8.12	6.65	8.59	4.83	8.46	7.46	7.54	7.38
Day-16	36.43	30.98	38.33	32.72	33.95	30.23	42.23	34.98
1100 ppm F								
Day-4	2.26	1.92	2.97	2.29	5.35	2.96	2.09	2.83
Day-8	4.08	4.23	7.44	5.67	8.07	5.90	4.92	5.76
Day-12	10.44	6.70	8.23	6.90	6.81	6.69	7.89	7.67
Day-16	67.26	38.26	41.68	40.71	44.21	37.11	34.88	43.44

Appendix 25: Poster presented at ORCA 2007 congress, Copenhagen, Denmark.

Effects of fluoridated Toothpaste on enamel surface loss progression *in vitro*

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GlaxoSmithKline

Overview

In a 21-day erosion/ abrasion *in vitro* cycling model a currently marketed low abrasion, de-sensitising toothpaste containing 1426ppm NaF demonstrated significantly less enamel surface loss than a matched placebo (0ppm F) and currently marketed de-sensitising toothpaste control (1500ppm MFP).

Introduction

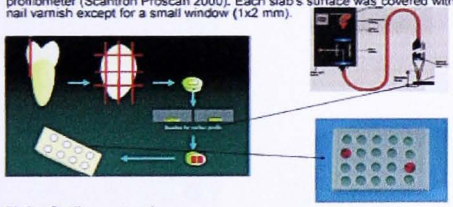
It has been suggested that the prevalence of tooth wear and associated hypersensitivity are likely to be on the increase[1]. Modern dietary habits frequently expose oral hard tissues to acidic substances leading to demineralisation and surface softening[2]. In this state they are more susceptible to mechanical wear from factors such as abrasion and attrition resulting in bulk tissue loss[3]. The mechanisms by which fluoridated toothpastes have helped to reduce dental caries is well-characterised[4-5] and therefore it seems reasonable to consider a role for them in the prevention and early management of erosive lesions. A longitudinal *in vitro* model has been developed to help characterise the effects of toothpaste formulations on tooth surface loss.

Objectives

To investigate the effects of two fluoridated toothpastes and a non-fluoridated control on enamel surface loss in a longitudinal *in vitro* erosion/ abrasion cycling model.

Methods

Slab preparation:
Three groups of eight human enamel slabs were mounted into resin blocks, ground and checked for surface flatness using a non-contact scanning profilometer (Scantron Proscan 2000). Each slab's surface was covered with nail varnish except for a small window (1x2 mm).

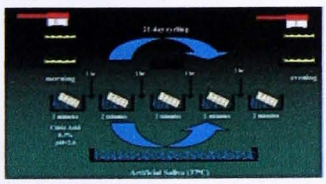


21-day Cycling protocol:

• Each group of resin blocks were immersed in a 1:3 toothpaste slurry and brushed, morning and evening, for 2 minutes at 200g weight in one of 3 different toothpastes:

- Toothpaste A: 1426ppm NaF+ 5% potassium nitrate; RDA 34 ± 2
- Toothpaste B: 1500ppm MFP + 5.53% potassium citrate; RDA 204 ± 5
- Toothpaste C: Matched placebo (0ppm F); RDA 33 ± 3

• Groups were immersed under static conditions for 2 minutes, five times daily in fresh 200 ml aliquots of citric acid 0.3% (pH=3.6). A sixty minute gap was left between daytime erosive challenges.



• Slabs were incubated overnight and between erosive challenges in artificial saliva at 37°C.
• Slabs were analysed with the scanning profilometer to measure the amount of surface loss at days 8, 12, 14, 16 and 21.

Statistical Analysis:

An SPSS statistical software package for Windows, version 13.0 (SPSS Inc., 1989-2002; LEAD technologies Inc., 1991-2000) was used for data analysis. Significance was considered using '*P*' value of less than 0.05 or a confidence interval not included "ZERO" value when the groups were compared.

Results

• Significant ($p<0.0001$) surface loss was observed in all treatment groups at all timepoints

• The extent of surface loss observed for paste A was statistically significantly less than pastes B and C at 12,14,16 and 21 day timepoints

• Toothpaste B demonstrated significantly less surface loss at day 21 than paste C. No other significant differences were observed between pastes B and C

Figure 1 Surface Loss Progression of Enamel Specimens

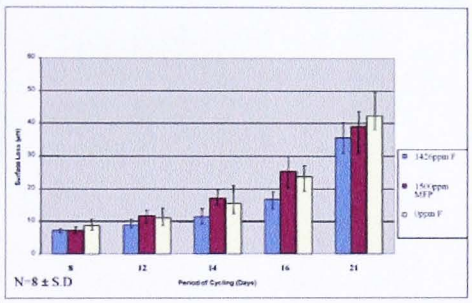


Table 1 Comparison of Mean Enamel Surface Loss

Brushing Groups		Difference of Means	SD	95% Confidence Interval of the Difference		P value (2-tailed)
				Lower	Upper	
Day 8	Toothpaste(A) VS Toothpaste(B)	0.01	0.65	-0.54	0.55	0.98
	Toothpaste(A) VS Toothpaste(C)	-1.43	1.06	-2.32	-0.55	0.01*
	Toothpaste(B) VS Toothpaste(C)	-1.44	0.80	-2.11	-0.77	0.00*
Day 12	Toothpaste(A) VS Toothpaste(B)	-2.68	1.34	-4.00	-1.76	0.00*
	Toothpaste(A) VS Toothpaste(C)	-2.23	2.28	-4.13	-0.32	0.03*
	Toothpaste(B) VS Toothpaste(C)	0.65	2.16	-1.17	2.47	0.43
Day 14	Toothpaste(A) VS Toothpaste(B)	-5.43	1.50	-6.66	-4.17	0.00*
	Toothpaste(A) VS Toothpaste(C)	-4.06	2.24	-5.93	-2.20	0.00*
	Toothpaste(B) VS Toothpaste(C)	1.36	2.81	-0.99	3.71	0.21
Day 16	Toothpaste(A) VS Toothpaste(B)	-8.39	3.30	-11.15	-5.63	0.00*
	Toothpaste(A) VS Toothpaste(C)	-6.85	2.96	-9.33	-4.37	0.00*
	Toothpaste(B) VS Toothpaste(C)	1.54	3.72	-1.57	4.65	0.28
Day 21	Toothpaste(A) VS Toothpaste(B)	-3.52	3.36	-6.33	-0.72	0.02*
	Toothpaste(A) VS Toothpaste(C)	-8.71	4.93	-16.83	-2.58	0.01*
	Toothpaste(B) VS Toothpaste(C)	-3.19	4.07	-6.59	0.21	0.06

Discussion

The significant differences in surface loss observed between paste A and its matched placebo support previous findings indicating that fluoridated toothpastes can offer protection to enamel from erosive challenges[6,7]. The results also suggest a role for toothpaste abrasivity in mediating surface loss progression. However the model did not contain the biological factors necessary for MFP hydrolysis and therefore the it is difficult to speculate on the relative performance of pastes A and B *in vivo*.

Conclusions

In this 21-day erosion/ abrasion cycling model a low abrasivity, desensitising 1426ppm sodium fluoride toothpaste significantly reduced the extent of enamel surface loss compared to a desensitising 1500ppm MFP paste and matched placebo control.

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Appendix 26: Poster presented at ORCA 2007 congress, Copenhagen, Denmark.

Leeds Dental Institute
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Erosion and abrasion effects on dentine surface loss *in vitro*

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Introduction

Tooth wear is a multi-factorial problem generally initiated following dissolution of mineralised tooth structure after contact with acids that are introduced into the oral cavity from Intrinsic (e.g., gastroesophageal reflux, vomiting) or extrinsic sources (e.g., acidic beverages, citrus fruits)[1]. In dentine, erosion progression was found to be inhibited by the presence of fluoride in vitro[2]. However, there are few data in the literature reporting the longitudinal effect of fluoride on dentine surface loss under erosive challenge mimicking the oral environment in vitro.

Study Aim

To investigate in vitro the effects of two fluoridated toothpastes and a non-fluoridated control on dentine surface loss erosion/ abrasion cycling model In a randomised, blinded experiment.

Materials and Methods

Slab preparation:
Three groups of eight human dentine slabs were cut from the buccal surface of intact human premolars. Then slabs were mounted into resin blocks, ground and checked for surface flatness using a non-contact scanning profilometer (Scantron Proscan 2000). Then, slabs were checked for surface harness using knoop microhardness (KMH) by indenting the slabs surface. Five indents length were used to check the hardness each slabs. Slabs included had an average length indents of 80-95 µm. Thereafter, each slab's surface was covered with nail varnish except for a small window (1x2 mm).

21-day Cycling protocol:
Each group of resin blocks were immersed in a 1:3 toothpaste slurry and brushed, morning and evening, for 2 minutes at 200g weight in one of 3 different toothpastes.
1. Toothpaste A: 1426ppm NaF+ 5% potassium nitrate; RDA 34 ± 2.
2. Toothpaste B: 1500ppm MFP + 5.53% potassium citrate; RDA 204 ± 5.
3. Toothpaste C: Matched placebo (0ppm F); RDA 33 ± 3.
The total cycling period lasted 21 days. Slabs were incubated overnight and between erosive challenges in artificial saliva at 37°C. A sixty minute gap was left between daytime erosive challenges. Slabs were analysed with the scanning profilometer to measure the amount of surface loss at days 8, 12, 14, 16 and 21.
Statistical Analysis:
An SPSS statistical software package for Windows, version 13.0 (SPSS Inc., 1989-2002; LEAD technologies Inc., 1991-2000) was used for data analysis. Significance was considered using "*P*" value of less than 0.05 or a confidence interval not included "ZERO" value when the groups were compared.

Results

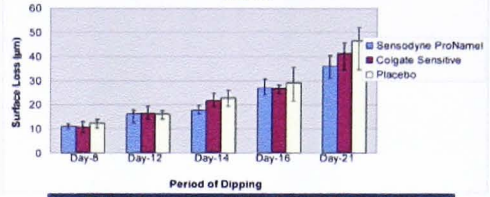
All toothpastes showed an increase in tooth surface loss from baseline. Toothpaste A showed less tooth surface loss than both B & C toothpastes at all timepoints. However, Statistical significance was observed only between fluoridated toothpastes and the control group.

Discussion

The results supports previous findings[2,3] about the effect of fluoride treatment in the reduction of the amount of tooth surface loss of dentine. However, in this study different cycling methodology was used.
The amount of fluoride concentration between toothpaste A is less than toothpaste B, however it has more inhibition affect on tooth surface loss. This might be due to the amount of silica in toothpaste B that would explain the superiority of toothpaste A regardless the superiority of toothpaste B in the concentration of fluoride. However, in the in vivo scenario, the situation is different because of the presence of biological factors that are required for MFP hydrolysis. Therefore more investigation is required to confirm these results in situ or in vivo.

Figure 1 Surface Loss Progression of Dentine Specimens

Comparison of the effect of 3 different concentrations of fluoridated toothpastes on human dentine using dipping with brushing twice daily in the toothpastes and using pH cycling technique (citric acid 0.3% , pH=3.6) for a period of 21 days (sample size of each group is 8)



Period of Dipping

Table 1 Comparison of Mean Dentine Surface Loss

Brushing Groups		Difference of Means (µm)	SD	95% Confidence Interval of the Difference		P value (2-tailed)
				Lower	Upper	
Day-8	Toothpaste(A) VS Toothpaste(B)	0.32	1.09	-0.59	1.23	0.43
	Toothpaste(A) VS Toothpaste(C)	-1.23	1.28	-2.30	-0.16	0.03*
	Toothpaste(B) VS Toothpaste(C)	-1.55	1.71	-2.98	-0.12	0.04*
Day-12	Toothpaste(A) VS Toothpaste(B)	-0.39	1.85	-1.94	1.16	0.57
	Toothpaste(A) VS Toothpaste(C)	-0.05	2.52	-2.15	2.06	0.96
	Toothpaste(B) VS Toothpaste(C)	0.34	2.02	-1.34	2.03	0.65
Day-14	Toothpaste(A) VS Toothpaste(B)	-4.01	1.80	-5.52	-2.51	0.00*
	Toothpaste(A) VS Toothpaste(C)	-5.24	2.43	-7.27	-3.21	0.00*
	Toothpaste(B) VS Toothpaste(C)	-1.23	2.88	-3.63	1.18	0.27
Day-16	Toothpaste(A) VS Toothpaste(B)	0.26	1.63	-1.08	1.64	0.64
	Toothpaste(A) VS Toothpaste(C)	-2.21	5.16	-6.53	-2.11	0.27
	Toothpaste(B) VS Toothpaste(C)	-2.49	4.77	-6.47	1.49	0.16
Day-21	Toothpaste(A) VS Toothpaste(B)	-5.11	5.33	-9.57	-0.66	0.03*
	Toothpaste(A) VS Toothpaste(C)	-10.47	5.83	-15.34	-5.60	0.00*
	Toothpaste(B) VS Toothpaste(C)	-5.36	7.94	-12.00	1.28	0.10

*statistically significant

Conclusion

In this modified cycling model a 1426ppm NaF dentifrice demonstrated significantly less enamel surface loss after 21 days than both the matched placebo and the 1500ppm MFP control dentifrice.

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Appendix 27: The effect of three different concentrations of fluoride toothpastes on human enamel using dipping/ dipping with brushing (brushing machine) twice daily in the toothpaste and using a pH cycling technique (0.3% citric acid pH=3.6) for a period of 21 days.

1450 ppm F+Brushing + Artificial Saliva-1									
	Slab-1	Slab-2	Slab-3	Slab-4	Slab-5	Slab-6	Slab-7	Slab-8	Mean
Day-8	7.21	7.44	6.63	7.56	7.74	7.00	7.65	7.32	7.32
Day-12	8.78	8.70	8.65	8.62	8.85	10.66	8.20	8.92	8.92
Day-14	10.61	14.05	11.33	9.46	9.19	13.62	12.51	11.54	11.54
Day-16	16.13	16.12	17.56	17.22	13.99	17.55	18.97	16.84	16.79
Day-21	31.84	36.53	40.15	30.80	32.85	39.35	37.17	35.51	35.53
1450 ppm F-Brushing + Artificial Saliva-1									
	Slab-1	Slab-2	Slab-3	Slab-4	Slab-5	Slab-6	Slab-7	Slab-8	Mean
Day-8	6.82	6.14	7.00	6.35	6.06	7.33	7.25	6.91	6.73
Day-12	7.97	8.37	7.99	8.37	8.48	11.94	11.70	7.48	9.04
Day-14	9.08	10.77	13.71	13.87	13.10	17.63	14.57	11.80	13.07
Day-16	14.55	14.23	16.23	19.32	19.43	20.05	20.32	15.08	17.40
Day-21	23.23	33.03	27.38	31.73	22.15	29.42	31.51	36.91	29.42
1500 ppm F+Brushing + Artificial Saliva-1									
	Slab-1	Slab-2	Slab-3	Slab-4	Slab-5	Slab-6	Slab-7	Slab-8	Mean
Day-8	6.21	7.00	7.11	8.30	7.38	7.54	7.11	7.85	7.31
Day-12	9.58	10.73	12.11	11.72	10.90	13.01	12.94	13.44	11.80
Day-14	15.82	17.51	15.08	15.70	15.08	17.91	19.65	18.96	17.13
Day-16	21.65	20.38	22.24	25.00	23.78	30.03	29.52	28.89	25.19
Day-21	30.76	39.32	43.62	40.17	39.95	41.49	40.91	36.17	39.05
1500 ppm F-Brushing + Artificial Saliva-1									
	Slab-1	Slab-2	Slab-3	Slab-4	Slab-5	Slab-6	Slab-7	Slab-8	Mean
Day-8	7.48	8.39	7.53	7.39	6.85	8.30	7.36	7.19	7.56
Day-12	9.65	11.01	9.73	10.38	8.74	9.56	9.34	9.39	9.72
Day-14	14.78	14.56	11.55	11.13	12.36	13.21	13.58	13.95	13.14
Day-16	19.00	18.38	15.24	17.49	15.79	17.98	19.72	20.89	18.06
Day-21	30.29	32.35	30.26	39.19	37.11	33.45	37.96	26.79	33.42
0 ppm F+Brushing + Artificial Saliva-1									
	Slab-1	Slab-2	Slab-3	Slab-4	Slab-5	Slab-6	Slab-7	Slab-8	Mean
Day-8	7.44	8.70	8.65	8.62	8.85	10.66	8.20	8.89	8.75
Day-12	9.42	14.20	11.26	11.28	10.51	8.70	12.63	11.22	11.15
Day-14	12.42	21.03	15.77	15.58	15.09	14.20	16.31	14.40	15.60
Day-16	19.27	23.26	22.78	23.90	25.80	20.92	26.98	26.29	23.65
Day-21	39.65	38.13	43.72	41.24	49.59	45.42	41.36	38.77	42.23
0 ppm F-Brushing + Artificial Saliva-1									
	Slab-1	Slab-2	Slab-3	Slab-4	Slab-5	Slab-6	Slab-7	Slab-8	Mean
Day-8	7.60	8.84	9.63	8.43	9.00	8.10	7.26	7.97	8.35
Day-12	10.79	11.68	12.96	10.83	13.08	12.45	11.78	12.03	11.95
Day-14	13.64	16.18	16.00	11.93	14.90	13.56	16.77	14.87	14.73
Day-16	22.00	22.99	26.15	19.79	22.71	17.40	20.75	18.32	21.26
Day-21	37.35	43.43	44.78	34.21	33.79	34.71	39.04	37.13	38.05
0 ppm F-Brushing + Artificial Saliva-2									
	Slab-1	Slab-2	Slab-3	Slab-4	Slab-5	Slab-6	Slab-7	Slab-8	Mean
Day-8	10.16	7.67	7.82	7.55	7.24	7.80	8.46	8.50	8.15
Day-12	11.54	8.83	9.05	10.78	10.54	10.62	11.93	11.13	10.55
Day-14	14.82	12.56	15.24	14.34	15.25	11.92	14.98	16.31	14.43
Day-16	21.39	16.10	22.70	18.87	17.48	15.84	20.98	21.42	19.35
Day-21	37.67	33.91	32.92	30.99	37.16	34.28	32.10	39.10	34.76

Appendix 28: The effect of three different concentrations of fluoride toothpastes on human dentine using dipping/dipping with brushing (brushing machine) twice daily in the toothpaste and using a pH cycling technique (0.3% citric acid pH=3.6) for a period of 21 days.

1450 ppm F+Brushing									
	Slab-1	Slab-2	Slab-3	Slab-4	Slab-5	Slab-6	Slab-7	Slab-8	Mean
Day-8	11.33	10.03	10.23	10.12	11.85	10.46	11.56	12.02	10.95
Day-12	16.15	16.24	16.01	12.41	15.95	16.76	16.19	17.95	15.96
Day-14	16.47	18.10	19.59	16.72	16.09	17.21	16.80	19.10	17.51
Day-16	27.58	25.21	27.76	25.75	25.97	30.51	23.89	28.31	26.87
Day-21	36.76	35.19	38.28	33.33	40.36	35.38	30.82	37.07	35.90
1450 ppm F-Brushing									
	Slab-1	Slab-2	Slab-3	Slab-4	Slab-5	Slab-6	Slab-7	Slab-8	Mean
Day-8	8.44	8.22	8.23	8.42	9.53	9.52	9.23	9.82	8.93
Day-12	14.26	14.06	14.56	12.97	14.34	13.46	11.13	12.24	13.38
Day-14	15.70	15.45	13.57	12.45	15.62	16.18	17.58	17.81	15.55
Day-16	17.80	19.61	16.99	16.89	20.32	16.99	21.61	22.77	19.12
Day-21	41.79	30.35	20.92	23.68	34.20	24.33	30.34	23.77	28.67
1500 ppm F+Brushing									
	Slab-1	Slab-2	Slab-3	Slab-4	Slab-5	Slab-6	Slab-7	Slab-8	Mean
Day-8	10.41	8.88	9.72	8.35	11.22	11.95	11.55	12.98	10.63
Day-12	19.36	13.85	16.65	15.26	15.27	16.74	15.98	17.64	16.34
Day-14	22.21	20.93	20.42	21.99	20.87	22.06	19.07	24.65	21.53
Day-16	27.33	25.08	26.31	24.28	27.00	28.09	26.68	27.99	26.59
Day-21	45.47	34.61	42.35	44.14	41.81	33.97	43.92	41.80	41.01
1500 ppm F-Brushing									
	Slab-1	Slab-2	Slab-3	Slab-4	Slab-5	Slab-6	Slab-7	Slab-8	Mean
Day-8	9.08	8.83	9.51	9.87	9.21	6.98	10.73	9.01	9.15
Day-12	11.75	14.18	14.17	14.71	11.54	13.51	15.76	13.67	13.66
Day-14	19.65	16.78	19.20	22.51	15.63	16.48	21.12	19.41	18.85
Day-16	19.89	20.23	22.65	21.88	16.44	18.56	21.37	20.83	20.23
Day-21	34.20	32.79	29.77	32.88	31.54	33.34	34.02	33.98	32.81
0 ppm F+Brushing									
	Slab-1	Slab-2	Slab-3	Slab-4	Slab-5	Slab-6	Slab-7	Slab-8	Mean
Day-8	10.80	11.24	10.38	12.68	10.44	11.45	10.75	11.25	11.12
Day-12	16.22	14.51	15.21	16.06	17.85	16.53	16.65	14.32	15.92
Day-14	21.92	18.86	19.79	21.34	19.89	20.72	22.42	16.52	20.18
Day-16	26.59	24.58	22.34	24.01	21.97	24.85	20.90	16.84	22.76
Day-21	34.33	32.70	35.56	33.93	39.39	35.27	31.47	32.43	34.38
0 ppm F-Brushing									
	Slab-1	Slab-2	Slab-3	Slab-4	Slab-5	Slab-6	Slab-7	Slab-8	Mean
Day-8	10.43	10.29	11.30	13.57	13.01	12.07	13.72	13.05	12.18
Day-12	17.02	14.47	14.60	17.09	16.45	16.81	17.47	14.13	16.00
Day-14	25.94	25.98	23.07	21.49	19.39	22.68	22.17	21.30	22.75
Day-16	29.89	30.02	35.47	26.56	33.59	26.90	28.55	21.67	29.08
Day-21	51.93	49.77	51.42	45.11	47.39	49.09	41.65	34.61	46.37

Appendix 29: Abstract of poster presented at IADR 2008 congress, Toronto, Canada

TITLE: Fluoride Effects on dentine surface loss under erosive cycling conditions

AUTHORS: AP Barlow[a], SC Mason[a], AZ Abdullah[b], SM Strafford[b], SJ Brookes[c], KJ Toumba[b], MS Duggal[b]

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Objectives: To compare the effect of 5 different concentrations of sodium fluoride in matched toothpastes on surface loss of human dentine in vitro using erosive cycling technique.

Methods: Five groups of eight dentine slabs each were cut and mounted into resin blocks, ground and checked for surface flatness using a scanning profilometer (Scantron Proscan 2000). The surface of each slab was covered with nail varnish except for a small window (1X2 mm). Using a randomised, blinded study design slabs were immersed for 2 minutes, five times daily in fresh 200 ml aliquots of 0.3% citric acid (pH=3.6). In addition, each group was immersed in one of five fluoridated (0, 250, 500, 1150, or 1450 ppm NaF) toothpaste slurries twice daily, morning and evening, for 2 minutes each time. Total cycling period lasted 21 days during which slabs were incubated overnight and between erosive challenges in artificial saliva (37°C). A sixty minute gap was left between day time immersions. Slabs were analysed with scanning profilometery to measure the amount of surface loss at day 7, 14, and 21.

Results: Significant bulk tissue loss was observed across all treatment groups at all timepoints. Surface loss ± SD of dentine at day 21 caused by cycling with 0, 250, 500, 1150, or 1450 ppm NaF toothpastes was 42± 6µm, 28± 2µm, 26± 5µm, 24± 2µm and 24± 3µm respectively.

Conclusion: Dentine surface loss was reduced significantly (p 0.05) with flu oride concentrations in toothpaste over 250 ppm.

This project was supported by GlaxoSmithKline

Appendix 30: Abstract of poster presented at ORCA 2008 congress, Groningen, the Netherlands.

Leeds Dental Institute
FACULTY OF MEDICINE AND HEALTH

UNIVERSITY OF LEEDS

The Effect of Fluoride on Tooth Surface Loss of Enamel under Erosive Cycling Challenge

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AZ Abdullah[a], SM Strafford[a], SJ Brookes[b], KJ Toumba[a], AP Barlow[c], SC Mason[c], MS Duggal[a]
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1 Introduction

Tooth wear is a multi-factorial problem generally initiated following dissolution of mineralised tooth structure after contact with acids that are introduced into the oral cavity from intrinsic (e.g., gastroesophageal reflux, vomiting) or extrinsic sources (e.g., acidic beverages, citrus fruits)[1]. Erosion considered as an increasing problem in Europe[2-5], it seems reasonable to consider a role for fluoride in the prevention and early management of erosive lesions. However, there are few data in the literature reporting the longitudinal effect of fluoridated toothpastes on enamel surface loss under erosive cycling challenge.

Study Aim

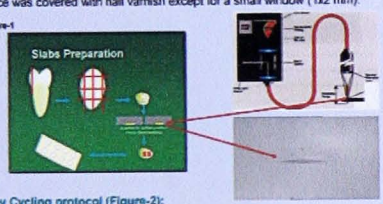
To investigate *in vitro* the effects of five matched toothpastes except in fluoride concentration on enamel surface loss in an erosion cycling model.

2 Materials and Methods

Slab preparation (Figure-1):

Five groups of eight human enamel slabs were cut from the buccal surface of intact human premolars. Slabs were mounted into resin blocks, ground and checked for surface flatness using a non-contact scanning profilometer (Scantron Proscan 2000). The slabs were checked for surface hardness using knoop microhardness (KMH) by indenting the slabs surface. Five indents length were used to check the hardness of each slabs. Slabs included had an average length indents of 80-95 µm. Thereafter, each surface was covered with nail varnish except for a small window (1x2 mm).

Figure-1



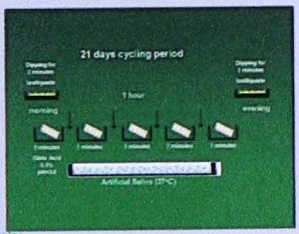
21-day Cycling protocol (Figure-2):

Each group of resin blocks were immersed in a 1:3 toothpaste slurry, morning and evening, for 2 minutes with one of 5 different toothpastes:

1. Toothpaste A: 0ppm F (as NaF);
2. Toothpaste B: 250ppm (as NaF);
3. Toothpaste C: 500ppm F (as NaF);
4. Toothpaste D: 1150ppm F (as NaF);
5. Toothpaste E: 1450ppm F (as NaF).

During cycling period, slabs were incubated overnight and between erosive challenges in artificial saliva at 37°C. A sixty minute gap was left between daytime erosive challenges. Slabs were analysed with the scanning profilometer to measure the amount of surface loss at days 7, 14, and 21.

Figure-2



Statistical Analysis:

An SPSS statistical software package for Windows, version 13.0 (SPSS Inc., 1989-2002; LEAD technologies Inc., 1991-2000) was used for data analysis. Treatment comparisons were done using paired t-test at 5% level of significance.

This Project was supported by GlaxoSmithKline

3 Results

All toothpastes showed an increase in tooth surface loss from baseline. However, a fluoride dose respond trend was seen. The amount of erosion was reduced with the increase of fluoride concentration. This decrease was statistically significant ($p \leq 0.05$) when 500ppm F toothpaste or higher fluoride toothpastes were used (Figure-3, Table-1).

Figure 3 Surface Loss Progression of Enamel Specimens

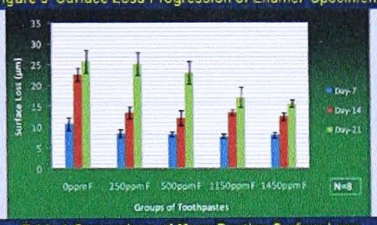


Table 1 Comparison of Mean Dentine Surface Loss

Day	Groups	Mean Diff (µm)	SE	Confidence Interval (95%) Min	Confidence Interval (95%) max	Sig.** (2-tailed)
Day - 7	Group 0ppm F vs Group 250ppm F	2.323	0.636	0.958	3.687	0.001*
	Group 0ppm F vs Group 500ppm F	2.490	0.585	1.235	3.745	0.001*
	Group 0ppm F vs Group 1150ppm	3.038	0.574	1.806	4.270	0.001*
	Group 0ppm F vs Group 1450ppm F	2.788	0.596	1.535	4.071	0.002*
	Group 250ppm F vs Group 500ppm F	0.166	0.383	-0.653	0.986	1.000
	Group 250ppm F vs Group 1150ppm	0.716	0.366	-0.069	1.500	0.093
	Group 250ppm F vs Group 1450ppm F	0.465	0.402	-0.397	1.328	0.945
	Group 500ppm F vs Group 1150ppm	0.548	0.258	-0.027	1.123	0.141
	Group 500ppm F vs Group 1450ppm F	0.298	0.316	-0.378	0.975	0.974
	Group 1150ppm F vs Group 1450ppm F	-0.250	0.295	-0.883	0.383	0.945
Day - 14	Group 0ppm F vs Group 250ppm F	9.291	0.756	7.670	10.913	0.001*
	Group 0ppm F vs Group 500ppm F	10.615	0.844	8.809	12.425	0.001*
	Group 0ppm F vs Group 1150ppm	9.354	0.608	8.049	10.659	0.001*
	Group 0ppm F vs Group 1450ppm F	10.278	0.641	8.904	11.652	0.001*
	Group 250ppm F vs Group 500ppm F	1.323	0.815	-0.425	3.072	0.093
	Group 250ppm F vs Group 1150ppm	0.063	0.567	-1.154	1.279	0.529
	Group 250ppm F vs Group 1450ppm F	0.987	0.632	-0.304	2.277	0.115
	Group 500ppm F vs Group 1150ppm	-1.260	0.680	-2.720	0.199	0.115
	Group 500ppm F vs Group 1450ppm F	-0.336	0.709	-1.856	1.185	0.945
	Group 1150ppm F vs Group 1450ppm F	0.924	0.400	0.065	1.783	0.040*
Day - 21	Group 0ppm F vs Group 250ppm F	0.657	1.370	-2.282	3.595	0.674
	Group 0ppm F vs Group 500ppm F	2.775	1.374	-0.172	5.722	0.048*
	Group 0ppm F vs Group 1150ppm	8.756	1.297	5.975	11.538	0.001*
	Group 0ppm F vs Group 1450ppm F	10.325	1.032	8.122	12.548	0.001*
	Group 250ppm F vs Group 500ppm F	2.119	1.354	-0.786	5.023	0.074
	Group 250ppm F vs Group 1150ppm	8.100	1.276	5.363	10.836	0.002*
	Group 250ppm F vs Group 1450ppm F	9.676	1.095	7.523	11.834	0.001*
	Group 500ppm F vs Group 1150ppm	5.981	1.280	3.236	8.726	0.002*
	Group 500ppm F vs Group 1450ppm F	7.560	1.010	5.398	9.726	0.001*
	Group 1150ppm F vs Group 1450ppm F	1.579	0.993	-0.357	3.515	0.115

*Statistically significant
** Bonn-Winding 'C test' was used to calculate "p" values

4 Discussion

The significant differences in surface loss observed between high fluoride concentration toothpaste (F ≥ 500ppm) and its matched placebo support previous findings indicating that fluoridated toothpastes can offer protection to enamel from erosive challenges[6-7].

Conclusion

In this modified erosion cycling model, there was a fluoride dose respond in reduction of erosion of enamel. This effect was statistically significant when toothpastes with a fluoride concentration of 500ppm F or higher were used.

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Appendix 31: Enamel Surface Loss after using 21 days cycling period and five matched toothpastes except in the amount of fluoride concentration as preventative agent.

	Slab no.	Day-7	Day-14	Day-21
1450 ppm F	1	6.85	12.76	15.39
	2	8.20	14.10	15.24
	3	7.28	11.74	14.88
	4	8.20	11.48	15.05
	5	9.01	12.04	16.09
	6	8.43	13.44	17.39
	7	8.00	12.16	14.53
	8	7.68	12.09	15.41
	Mean	7.96	12.48	15.50
	Slab no.	Day-7	Day-14	Day-21
0 ppm F	1	8.67	20.59	25.97
	2	12.63	22.84	22.66
	3	8.90	21.79	29.39
	4	11.00	22.87	29.77
	5	9.90	22.63	26.03
	6	11.11	21.38	22.44
	7	10.87	25.05	23.87
	8	12.89	24.88	26.53
	Mean	10.74	22.75	25.83
	Slab no.	Day-7	Day-14	Day-21
250 ppm F	1	7.89	15.44	25.07
	2	8.65	12.83	25.04
	3	7.44	12.22	26.33
	4	8.47	12.55	25.52
	5	8.07	13.08	28.95
	6	8.84	13.90	26.18
	7	7.68	11.92	25.00
	8	10.34	15.76	19.32
	Mean	8.42	13.46	25.18
	Slab no.	Day-7	Day-14	Day-21
500 ppm F	1	7.36	14.24	15.42
	2	8.21	13.11	19.19
	3	7.97	13.28	15.22
	4	6.79	13.38	13.14
	5	8.20	12.38	17.20
	6	8.01	14.56	17.04
	7	7.55	13.00	19.47
	8	7.56	13.25	19.94
	Mean	7.71	13.40	17.08
	Slab no.	Day-7	Day-14	Day-21
1150 ppm F	1	8.13	11.99	23.55
	2	8.98	14.91	22.39
	3	7.52	14.63	25.89
	4	8.13	11.03	25.55
	5	8.72	10.21	21.26
	6	7.67	11.34	17.50
	7	7.88	12.56	23.81
	8	9.01	10.44	24.49
	Mean	8.25	12.14	23.06

Appendix 32: Dentine Surface Loss after using 21 days cycling period and five matched toothpastes except in the amount of fluoride concentration as preventative agent.

	Slab no.	Day-7	Day-14	Day-21
1450 ppm F	1	9.684	16.130	24.047
	2	9.107	17.504	26.683
	3	9.906	16.640	27.742
	4	13.769	14.697	21.798
	5	10.659	15.961	22.923
	6	9.796	16.307	25.433
	7	8.760	17.582	26.917
	8	10.234	16.493	19.183
	Mean	10.239	16.414	24.341
	Slab no.	Day-7	Day-14	Day-21
0 ppm F	1			
	2	12.392	28.540	34.434
	3	13.589	27.417	35.145
	4	11.414	29.731	38.890
	5	14.998	24.371	34.713
	6	11.944	24.520	48.213
	7	14.595	25.993	41.598
	8	13.500	29.346	50.581
	Mean	13.314	26.828	41.155
	Slab no.	Day-7	Day-14	Day-21
250 ppm F	1	9.470	9.740	26.150
	2	11.986	16.890	29.132
	3	8.408	18.973	29.526
	4	10.962	12.717	25.423
	5	9.742	22.123	25.993
	6	12.615	20.085	30.212
	7	11.101	19.201	28.581
	8	12.001	22.115	28.809
	Mean	10.786	17.731	27.978
	Slab no.	Day-7	Day-14	Day-21
500 ppm F	1	10.771	15.663	22.713
	2	8.262	17.627	25.043
	3	12.050	17.660	26.520
	4	12.246	13.634	25.221
	5	6.534	18.824	19.703
	6	10.078	12.907	23.001
	7	11.518	15.269	24.046
	8	10.125	17.386	25.688
	Mean	10.198	16.121	23.992
	Slab no.	Day-7	Day-14	Day-21
1150 ppm F	1	10.419	13.223	28.161
	2	9.087	12.745	26.691
	3	11.112	17.888	30.237
	4	10.571	11.424	19.835
	5	10.655	14.482	26.659
	6	10.654	16.377	19.277
	7	9.509	17.878	29.241
	8	10.419	13.223	28.161
	Mean	9.087	12.745	26.691

Appendix 33: The effect of three different RDA content in toothpastes on the amount of enamel surface loss caused by using a 21-day pH cycling technique.

140 RDA Slab no.	Day-7	Day-14	Day-21
1	8.687	14.530	22.938
2	9.780	13.533	20.501
3	10.927	15.693	25.695
4	8.907	15.112	23.289
5	8.846	13.092	16.415
6	6.906	18.954	16.176
7	11.736	13.780	22.319
8	11.552	15.795	16.077
Mean	9.668	15.061	20.426
160 RDA Slab no.	**Day-7**	**Day-14**	**Day-21**
1	8.932	14.561	20.007
2	9.999	12.158	16.915
3	9.130	13.887	24.222
4	7.947	11.048	22.189
5	8.776	15.873	16.433
6	10.561	14.450	19.032
7	9.138	18.021	22.456
8	8.930	11.782	19.893
Mean	9.177	13.973	20.143
40 RDA Slab no.	**Day-7**	**Day-14**	**Day-21**
1	7.556	14.657	16.860
2	8.132	16.328	18.630
3	7.947	15.790	18.440
4	7.987	15.155	19.283
5	8.686	11.330	23.484
6	7.446	13.620	16.653
7	7.075	10.942	18.587
8	6.908	10.522	16.310
Mean	7.717	13.543	18.531

Appendix 34: The effect of three different RDA content in toothpastes on the amount of enamel surface loss caused by using a 21-day pH cycling technique.

140 RDA Slab no.	Day-7	Day-14	Day-21
1	10.718	15.569	25.810
2	10.609	13.062	25.491
3	9.494	16.480	28.207
4	10.495	18.030	26.621
5	11.591	17.507	30.101
6	10.515	13.793	27.589
7	10.340	18.329	28.486
8	10.583	20.052	28.221
Mean	10.543	16.603	27.566
160 RDA Slab no.	**Day-7**	**Day-14**	**Day-21**
1	10.079	14.259	24.465
2	9.854	14.361	27.149
3	10.876	20.277	26.476
4	10.187	18.504	26.134
5	12.102	12.402	26.301
6	9.525	18.340	24.417
7	9.883	12.692	24.460
8	10.878	16.181	23.569
Mean	10.423	15.877	25.371
40 RDA Slab no.	**Day-7**	**Day-14**	**Day-21**
1	12.640	17.517	25.373
2	11.333	15.031	28.499
3	10.122	14.582	25.509
4	6.726	18.090	24.452
5	10.940	14.915	26.490
6	12.288	17.611	25.195
7	8.686	15.728	25.603
8	11.230	14.870	26.503
Mean	10.496	16.043	25.953

Appendix 35: Abstract that was presented at EAPD 2008 congress, Dubrovnik, Croatia.

Title: Comparison of the Effect of a Longitudinal Erosive Challenge on Permanent and Deciduous Enamel

AZ Abdullah[a*], SM Strafford[a], SJ Brookes[a], KJ Toumba[a], SC Shirodaria[b], AP Barlow[b], SR Smith[b], MS Duggal[a]. [a]Leeds Dental Institute, UK, [b]GlaxoSmithKline, UK. den2aa@leeds.ac.uk

Objectives: To compare the effect of toothpastes with different fluoride concentrations on tooth surface loss of human enamel in vitro in an erosive model. **Methods:** In a randomised, blinded experiment 6 groups (8 slabs/group) each of deciduous and permanent enamel slabs were used. The groups were Sensodyne Pronamel children formula (CF) with 0, 500, 1000, 1426ppm NaF, Sensodyne Pronamel adult formula (AF) with 1426ppm NaF, and Sensodyne Pronamel (CF) 1426ppm NaF with one application of 2 minutes per week of high fluoride gel (Project Max) 2500ppm NaF. A cycling regime of 21 days was used. The slabs were immersed under static conditions for 2 minutes, five times daily in fresh 200 ml aliquots of citric acid 0.3% (pH=3.6). In addition, each group was dipped with one of the toothpastes twice daily morning and evening, for 2 minutes each time. The slabs were incubated overnight and between erosive challenges in artificial saliva at 37°C. The slabs were analysed with a scanning profilometer (Scantron Proscan 2000) to measure the amount of surface loss at day 7, 14, and 21. **Results:** Surface loss (μm) ± SD of deciduous enamel at day 21 using CF with 0, 500, 1000, 1426, 1426+Project Max, and AF with 1426ppm NaF toothpastes was 24.58±1.84, 20.37±1.43, 18.85±4.78, 17.12±1.94, 16.85±1.46, and 16.31±2.84 respectively. In a similar order, surface loss (μm) of permanent enamel at day 21 was 25.08±1.49, 19.14±1.65, 16.56±3.50, 14.71±1.44, 14.21±2.00, and 14.83±1.81. **Conclusion:** All enamel showed significantly less surface loss when fluoride toothpastes were used. In addition, deciduous enamel showed more surface loss than permanent enamel but this was not significant in all groups. **Sponsor:** GlaxoSmithKline.

Appendix 36: Tooth surface loss of deciduous enamel after exposure for 21 erosive challenge using modified cycling technique and using six different fluoride treatment.

		500 ppm F	1426 ppm F	0 ppm F	1000 ppm F	1450 ppm F + HFG	1450 ppm F (MC)
7-Days	Slab-1	2.18	3.88	5.33	3.02	3.23	4.59
	Slab-2	4.95	5.93	2.92	4.60	2.44	3.28
	Slab-3	2.62	2.04	3.17	3.15	3.55	5.42
	Slab-4	2.61	3.35	4.25	4.25	2.54	4.90
	Slab-5	3.70	3.32	3.20	6.53	3.87	4.43
	Slab-6	3.98	3.42	11.68	9.87	9.69	4.13
	Slab-7	2.76	2.39	4.08	6.98	4.19	2.19
	Slab-8	7.95	3.34	7.37	2.08	2.29	3.20
	Mean	3.84	3.46	5.25	5.06	3.97	4.02
		500 ppm F	**1426 ppm F**	**0 ppm F**	**1000 ppm F**	**1450 ppm F + HFG**	**1450 ppm F (MC)**
14-Days	Slab-1	9.72	8.66	13.84	8.26	7.28	16.28
	Slab-2	12.06	12.21	11.87	9.67	7.78	9.15
	Slab-3	8.58	7.02	10.78	7.66	9.14	9.11
	Slab-4	8.24	5.79	12.24	10.13	4.43	10.11
	Slab-5	14.71	7.40	13.42	21.22	9.70	10.48
	Slab-6	10.11	6.64	9.30	29.21	7.48	9.96
	Slab-7	10.20	5.82	15.00	9.62	10.99	8.84
	Slab-8	8.17	5.09	7.50	7.05	6.91	10.86
	Mean	10.22	7.33	11.74	12.85	7.96	10.60
		500 ppm F	**1426 ppm F**	**0 ppm F**	**1000 ppm F**	**1450 ppm F + HFG**	**1450 ppm F (MC)**
21-Days	Slab-1	22.73	15.00	22.06	14.36	15.57	16.47
	Slab-2	18.91	18.59	24.27	19.64	18.98	18.11
	Slab-3	20.31	13.92	26.82	15.88	16.44	19.31
	Slab-4	19.45	16.68	26.18	16.58	18.89	17.58
	Slab-5	21.36	18.01	25.75	22.37	15.28	13.36
	Slab-6	21.80	18.06	25.84	28.18	17.49	12.45
	Slab-7	19.23	19.87	22.26	13.91	15.70	13.54
	Slab-8	19.20	16.83	23.43	19.87	16.46	19.64
	Mean	20.37	17.12	24.58	18.85	16.85	16.31

Appendix 37: Tooth surface loss of permanent enamel after exposure for 21 erosive challenge using modified cycling technique and using six different fluoride treatment.

		500 ppm F	1426 ppm F	0 ppm F	1000 ppm F	1450 ppm F + HFG	1450 ppm F (MC)
7-Days	Slab-1	3.01	3.72	3.74	2.11	2.71	3.05
	Slab-2	3.30	2.11	6.85	2.47	6.66	3.15
	Slab-3	3.64	2.27	3.65	2.24	2.05	2.99
	Slab-4	2.27	2.71	3.14	2.79	2.05	3.31
	Slab-5	2.98	6.05	5.70	2.33	2.05	3.30
	Slab-6	3.58	2.13	3.30	2.93	2.71	2.95
	Slab-7	2.47	4.40	3.14	3.04	2.96	3.82
	Slab-8	3.25	3.77	3.73	2.59	3.66	3.01
	Mean	3.06	3.39	4.16	2.56	3.10	3.20
		500 ppm F	**1426 ppm F**	**0 ppm F**	**1000 ppm F**	**1450 ppm F + HFG**	**1450 ppm F (MC)**
14-Days	Slab-1	9.25	7.74	10.35	10.39	7.97	9.68
	Slab-2	10.51	8.69	14.11	5.65	9.92	8.86
	Slab-3	10.96	12.20	9.88	10.00	7.74	9.16
	Slab-4	9.58	7.64	9.63	5.65	8.06	8.19
	Slab-5	7.53	8.52	10.51	5.42	7.25	8.14
	Slab-6	9.57	5.96	11.16	6.19	8.32	9.61
	Slab-7	9.25	8.63	10.67	7.11	7.95	7.01
	Slab-8	8.33	6.70	13.99	5.12	7.99	6.53
	Mean	9.37	8.26	11.29	6.94	8.15	8.40
		500 ppm F	**1426 ppm F**	**0 ppm F**	**1000 ppm F**	**1450 ppm F + HFG**	**1450 ppm F (MC)**
21-Days	Slab-1	19.98	16.70	26.37	11.69	15.91	17.50
	Slab-2	18.64	13.62	23.76	10.40	12.08	14.30
	Slab-3	18.00	12.15	26.17	19.86	15.26	12.19
	Slab-4	19.95	14.45	25.44	18.19	11.56	14.07
	Slab-5	17.06	14.15	23.70	18.00	15.31	13.43
	Slab-6	22.13	15.30	26.45	18.81	12.30	16.95
	Slab-7	17.57	15.23	26.08	18.32	14.29	15.93
	Slab-8	19.84	16.05	22.64	17.20	16.99	14.30
	Mean	19.14	14.71	25.08	16.56	14.21	14.83

Appendix 38: Ethical Approval

NHS
National Research Ethics Service
Leeds (West) Research Ethics Committee
A/B Floor, Old Site
Leeds General Infirmary
Great George Street
Leeds
LS1 3EX

Telephone: 0113 3923181
Facsimile: 0113 3923803

12 October 2007

Mr Ahmed Abdullah
PhD Student
Leeds Dental Institute
Paediatric Dentistry Department
Level 8, Worsley Building
Leeds
LS2 9LU

Dear Mr Abdullah

Full title of study: The Effect of Fluoridated Toothpastes on Surface Loss of the Dental Hard Tissues under Erosive Conditions

REC reference number: 07/H1307/136

Thank you for your letter of 10 October 2007, responding to the Committee's request for further information on the above research and submitting revised documentation.

The further information has been considered on behalf of the Committee by the Chair.

Confirmation of ethical opinion

On behalf of the Committee, I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the application form, protocol and supporting documentation as revised.

Ethical review of research sites

The Committee has designated this study as exempt from site-specific assessment (SSA). There is no requirement for [other] Local Research Ethics Committees to be informed or for site-specific assessment to be carried out at each site.

Conditions of approval

The favourable opinion is given provided that you comply with the conditions set out in the attached document. You are advised to study the conditions carefully.

Approved documents

The final list of documents reviewed and approved by the Committee is as follows:

Document	Version	Date
Application		20 July 2007
Investigator CV	Prof M E Duggal	

This Research Ethics Committee is an advisory committee to [illegible] and the Northern Ireland Ethics Committee
The National Research Ethics Service (NRES) represents the NRES Directorate within the National Patient Safety Agency and Research Ethics Committees in England

Investigator CV	Ahmed Abdullah	
Investigator CV	Polydoros Jack Tourvas	
Protocol		
Covering Letter		20 July 2007
Summary/Synopsis		
Advertisement		
Letter of invitation to participant		
GP/Consultant Information Sheets		
Participant Information Sheet	3	
Participant Consent Form		
Response to Request for Further Information		11 September 2007
Response to Request for Further Information	2	10 October 2007
Letter from GI for LTHT		03 October 2007
Letter from HTA Listener Holder		03 October 2007
Letter regarding LOI below form		11 September 2007

R&D approval

All researchers and research collaborators who will be participating in the research at NHS sites should apply for R&D approval from the relevant care organisation, if they have not yet done so. R&D approval is required, whether or not the study is exempt from SSA. You should advise researchers and local collaborators accordingly.

Guidance on applying for R&D approval is available from http://www.rdforum.nhs.uk/rdforum.htm.

Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees (July 2001) and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

Feedback on the application process

Now that you have completed the application process you are invited to give your view of the service you received from the National Research Ethics Service. If you wish to make your views known please use the feedback form available on the NRES website at:

https://www.nresform.org.uk/AppFormModules/Feedback/EthicalReview.aspx

We value your views and comments and will use them to inform the operational process and further improve our service.

07/H1307/136	Please quote this number on all correspondence

With the Committee's best wishes for the success of this project

Yours sincerely

07/H1307/136

F

Laura Sawiuk
REC Co-ordinator
On Behalf of
Mr Jon Silcock
Chair

Email: laura.sawiuk@leedsth.nhs.uk

Enclosures: *Standard approval conditions*

Copy to: Mrs Claire Skinner, University of Leeds

R&D, LTHT

Appendix 39 Research and Development Department approval

The Leeds Teaching Hospitals NHS
NHS Trust

Research & Development Directorate
A/8 Corridor, Old Site
The General Infirmary at Leeds
Great George Street
Leeds
West Yorkshire
LS1 3EX

Tel: 0113 392 2878
Fax: 0113 392 6397

www.leedsteachinghospitals.com

11/12/2007

Mr Ahmed Abdullah
Paediatric Dentistry Department
Level 6
Worsley Building
Leeds
LS2 9LU

Dear Mr Ahmed Abdullah

Re: LTHT R&D Approval of DT07/8297: The effect of fluoridated toothpastes on surface loss of the dental hard tissues under erosive conditions

I write with reference to the above research study. I can now confirm that this study has R&D approval and the study may proceed at The Leeds Teaching Hospitals NHS Trust (LTHT). This organisational level approval is given based on the information provided in the documents listed below.

As principal investigator you have responsibility for the design, management and reporting of the study. In undertaking this research you must comply with the requirements of the *Research Governance Framework for Health and Social Care* which is mandatory for all NHS employees. This document may be accessed on the Department of Health website at http://www.dh.gov.uk/research

R&D approval is therefore given 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

The Leeds Teaching Hospitals NHS Trust participates in the NHS risk pooling scheme administered by the NHS Litigation Authority 'Clinical Negligence Scheme for NHS Trusts' for: (i) medical professional and/or medical malpractice liability; and (ii) general liability. NHS Indemnity for negligent harm is extended to researchers with an employment contract (substantive or honorary) with the Trust. The Trust

Chairman Martin Buckley Chief Executive Maggie Boyle
The Leeds Teaching Hospitals incorporating: Chapel Allerton Hospital Cookridge Hospital Leeds Chest Clinic
Leeds Dental Institute Seacroft Hospital St James's University Hospital The General Infirmary at Leeds
Wharfedale Hospital

only accepts liability for research activity that has been managerially approved by the R&D Department.

The Trust therefore accepts liability for the above research project and extends indemnity for negligent harm to cover you as principal investigator and the researchers listed on the R&D approval form provided that each member of the research team has an employment contract (substantive or honorary) with the Trust. Should there be any changes to the research team please ensure that you inform the R&D Department and that s/he obtains an employment contract with the Trust if required.

Yours sincerely

Dr D R Norfolk
Associate Director of R&D

Appendix 40: Information sheets

Volunteer Information Sheet

Thank you for expressing an interest in our forthcoming study. Please find below some more information about the study, which we hope will answer some of the questions you may have.

Study Title:

The Effect of Copper on Remineralisation of the Dental Hard Tissues under Cariogenic Conditions.

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, and discuss it with others if you wish. Ask us if there is anything that is not clear, or if you would like more information. Take time to decide whether or not you wish to take part.

What is the purpose of the study?

The aim of this study is to evaluate the effect of copper on prevention of dental decay and compare that effect with the effect of fluoride. It is known that fluoride has an excellent effect on inhibition of dental decay. In our study we will try to find out about a new idea of preventing dental decay by using copper.

We will use four different solutions in our study and we will compare among their effects on prevention of dental decay. The solutions are copper; copper and fluoride; fluoride; and water.

On completion of the study period, we will offer to apply a topical fluoride to your teeth to promote remineralisation. Participating in these kinds of studies carries some risk of mineral loss from your teeth, mainly because of the omission of fluoride toothpaste.

Why have I been chosen?

We hope to recruit volunteers for this study. All we ask of volunteers is that they are willing to take part in the study; that they are at least 18 years old; that they are in general good health; that they are not pregnant or planning to become pregnant during the course of the study; that they have at least 18 natural teeth; and that they have some fillings. 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 out criteria. We will also ask you a few simple questions about your general health. You will also have the opportunity to ask us any questions you may have about the study.

Do I have to take part?

Participation in this study is entirely voluntary. If you decide you would like to take part, you will be given a copy of this information sheet to keep, and we will ask you

to complete and sign a form which gives your written consent to take part. If after reading this and thinking about the information given, you decide you would not like to take part

that is fine. 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, and you do not have to give us a reason unless you want to. If you do decide to withdraw from the study at any point, please let us reassure you that your future dental care at the Dental Hospital will in no way be compromised. If you are a member of staff of the Trust or University, you are under no obligation whatsoever to take part in this study, but if you decide you would like to take part, you can still withdraw at any point without having to give a reason.

What will happen to me if I take part?

If you decide you would like to take part, and our screening procedures identify you as a suitable volunteer, a removable device similar to an orthodontic plate will be constructed for you. You will have to wear this removable appliance which contains two sterilised enamel sections. These enamel sections are the parts that we will do our tests on. It will be given to you 2 days before each experiment and should be worn at all times, except at mealtimes and during teeth brushing times also. You will be provided with fluoride-free toothpaste, which you will use twice daily to brush your teeth normally but not to brush the appliance. We will show you how to remove and re-insert the device, and you will have ample opportunity to ask any questions, and to make sure you are happy and confident in using the device, before starting the study.

The study consists of 4 parts and each part will last one month, therefore the full study time will be 4 months. You need to visit us 4-5 times in each period, about once each week. These visits are to supply you with the device; solutions; and to collect it again by the end of that period.

What do I have to do?

You will need to agree to wear the device we will construct for you, and to agree to remove the device when eating and/or drinking. You will need to dip the device given to you in the solution provided to you as we will instruct you (seven times a day). You will need to use the special toothpaste we will give you. You will also need to agree to come into the test centre at the dates and times agreed. 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. In recognition of any inconvenience and out of pocket expenses you will incur, you will be paid a fee of £450 for taking part in the study. This money will be paid to you at the end of the study. In order for us to pay you, you will need to complete a bank details form, and provide us with your National Insurance Number. This information will be held confidentially.

What is the procedure that is being tested?

As mentioned in our introduction, we are developing a different way to measure the efficacy of copper on the inhibition of enamel decay. If we prove that this way of using the device works, we hope to set a precedent for other researchers.

What are the side effects of taking part?

We hope there will be no side effects whatever for volunteers. The only slight risk to you as a volunteer would 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. As an extra precaution, you will have a

topical fluoride gel professionally applied at the end of the study. However, we are very confident that no significant damage will be caused to you or your teeth if you take part in this study.

What are the possible disadvantages or risks of taking part?

The only disadvantage to you as a volunteer will be that you will be asked to wear and remove the device at the times specified. For the first few days of the study, you may find this to be a slight inconvenience. However, once you get into the routine of working with the device, we are sure you will find it easy to remember what to do and when.

What are the possible benefits of taking part?

There are no direct immediate benefits to you for participating in our study. However, you will be helping us develop what we hope will be a new way of studying copper and how it affects our teeth. This will ultimately help scientists develop new materials for dental caries prevention.

What if new information becomes available?

If any new information becomes available about either the devices or the test products during the study, we will of course let you know as soon as possible, and explain the changes to you. You will have the chance to ask us any questions before deciding whether you would like to continue with the study. If the changes are significant, it may be that we ask for your written consent to continue with the study. Please let us stress that, should this occur, you will be under no obligation whatsoever to continue with the study, and you may still withdraw at any point without giving a reason.

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, just incase any slight damage has been caused by your using a non-fluoride toothpaste. We will then collect all our data, and examine it in our laboratory. Any information we collect about you during our study will be kept strictly confidential.

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. If you feel that the matter is serious, it may be that you will be entitled to appropriate compensation, from the sponsors of the study (GABA). The sponsor, without legal commitment should compensate you without having to prove that it is at fault. This applies in cases where it is likely that such injury has resulted from any new drugs or procedures carried out in accordance with the protocol of the study. "The sponsor" will not compensate you where such injury results from any procedure which is not in accordance with the protocol for the study. Your right to law to claim compensation for the injury where you can prove negligence is not affected.

Will my taking part in this study be kept confidential?

If you consent to take part in this study, your medical/dental records may need to be accessed in certain instances, for the purpose of analysing our results. Any information we gather will be kept confidential. You will not be identified by name in any reports or

publications. However, under new guidelines in place for research projects, we will need to write to your dentist, just to let him/her know that you are taking part in this study, and to give them the opportunity to contact us for more information should they require it.

What will happen to the results of the research study?

We hope our research will be well received by the dental community. You will not be identified by name in any reports we write.

Who is organizing and funding the research?

The study is being carried out by the research team at the Leeds International Centre for Cariogenicity Research (LICCR). More specifically, the research will be carried out by the principal investigator Dr Ahmad Abdullah and supervised by Professor Monty Duggal, Dr Steve Brookes, and Dr. Simon Strafford. Professor Duggal is Head of Pediatric Dentistry, Dr. Simon Strafford is a research assistant at Paediatric Dentistry, and is overseeing this study. Dr Steve Brookes, a senior lecturer in Oral Biology Department. The study is being sponsored/funded by GABA. They are a toothpaste manufacturing company based in Basel, Switzerland.

Contact for further information:

If you would like any further information at all, either before or during the study, please do not hesitate to contact any one of us. Please find below our telephone numbers and e-mail addresses.

Name	Telephone	e-mail
Dr Ahmad Abdullah	(0113) 343 6195	den2aa@leeds.ac.uk
Professor Monty Duggal	(0113) 343 6177	m.s.duggal@leeds.ac.uk
Dr Steve Brookes	(0113) 233 6161	s.j.brookes@leeds.ac.uk
Research Coordinator	(0113) 233 6240	
(24hr Emergency Contact)	07717315457 / 07766 461 296	

Thank you for reading this

Appendix 41: consent

CONSENT FORM

The Effect of Fluoridated Toothpastes on Surface Loss of the Dental Hard Tissues under Erosive Condition

Screening No:.....

Please initial box

1. I confirm that I have read and understand the information sheet dated 20th July 2007 for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily. ☐
2. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my dental care or legal rights being affected. ☐
3. I understand that relevant sections of any of my dental notes and data collected during the study may be looked at by responsible individuals from study supervisors, from regulatory authorities or from the NHS Trust, where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records. ☐
4. I agree to my Dentist being informed of my participation in the study. ☐
5. I agree to take part in the above study. ☐

_____	_____	_____
Name of Patient	Date	Signature
_____	_____	_____
Researcher (Study Investigator)	Date	Signature

Appendix 42: randomisation table (in situ)

GlaxoSmithKline
Consumer Healthcare
Research and Development

[redacted]

Protocol Z2560489 Randomisation Schedule

Object name:	random_Z2560489
Status:	Issued
Version:	2.0
Object ID:	0900233c80586ba5
Issue date:	26-Sep-2007 08:18:49
Reason for issue:	New issue
Author(s):	Darren A Targett

Signed for approval by:	User ID:	Date (GMT):
Darren A Targett	targed00	26-Sep-2007 08:18:41

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Randomisation Number	Period 1	Period 2	Randomisation Number	Period 1	Period 2
0001	A	B	0028	B	A
0002	B	A	0029	A	B
0003	A	B	0030	B	A
0004	B	A	0031	B	A
0005	A	B	0032	A	B
0006	B	A	0033	A	B
0007	B	A	0034	B	A
0008	A	B	0035	A	B
0009	A	B	0036	B	A
0010	B	A	0037	B	A
0011	B	A	0038	A	B
0012	A	B	0039	A	B
0013	A	B	0040	B	A
0014	B	A	0041	A	B
0015	B	A	0042	B	A
0016	A	B	0043	A	B
0017	B	A	0044	B	A
0018	A	B	0045	A	B
0019	A	B	0046	B	A
0020	B	A	0047	B	A
0021	B	A	0048	A	B
0022	A	B	0049	B	A
0023	B	A	0050	A	B
0024	A	B	0051	A	B
0025	B	A	0052	B	A
0026	A	B	0053	A	B
0027	A	B	0054	B	A

Appendix 43: Case Record Form (One leg was included to avoid repetition).

Leeds Dental Institute

University of Leeds School of Dentistry with the

Dental Hospital at Leeds

Subject code:	Randomisation no.:	Screening no.:

Case Record Form

Study no: 07/H1307/136

The Effect of Fluoridated Toothpastes on Surface Loss of the Dental Hard Tissues under Erosive Condition

Study Investigator/Dentist

Dr Ahmed Abdullah

Division of Child Dental Health

Principal Investigators

Professor M. S. Duggal

Professor K. J. Toumba

Division of Child Dental Health

Volunteer Personnel Sheet

Personnel Information

First name:		Family name:
Date of birth: _ _ / _ _ / _ _ _ _		Marital status (Optional):
Gender: M ☐ F ☐		
Contact address:		
		Post code:
Tel:	Mobile:	e-mail:
Emergency contact		
Name		
Relationship		
Address:		
		Post code:
Tel:	Mobile:	e-mail:

Name:	Date:	Signature:

Official use only

Checked by:	Date:	Signature:
Subject code:	Randomisation no.:	

Subject code:	**Randomisation no.:**

MEDICAL HISTORY

Any medical conditions to report?	☐ YES	☐ NO

Please list any relevant previous and current medical conditions (including allergies) and surgery that the subject has experienced in the table below*.

MEDICAL CONDITION	START DATE (DD / MM / YYYY)	ONGOING	STOP DATE (IF APPLICABLE)
	...../...../.....	YES / NO	...../...../.....
	...../...../.....	YES / NO	...../...../.....
	...../...../.....	YES / NO	...../...../.....

***Note that if any treatment (s) is/are currently being taken for any of the above conditions this (these) must be recorded on the CURRENT / CONCOMITANT MEDICATIONS page.**

Signed by (Investigator).....

Print Name (Investigator).....

Dated (DD/MM/YY) ☐☐ ☐☐ ☐☐☐☐

Subject code:	Randomisation no.:

Current/ Concomitant medication

Drug's name	Cause of medication	Dosage	Date started	Date stopped (if applicable)

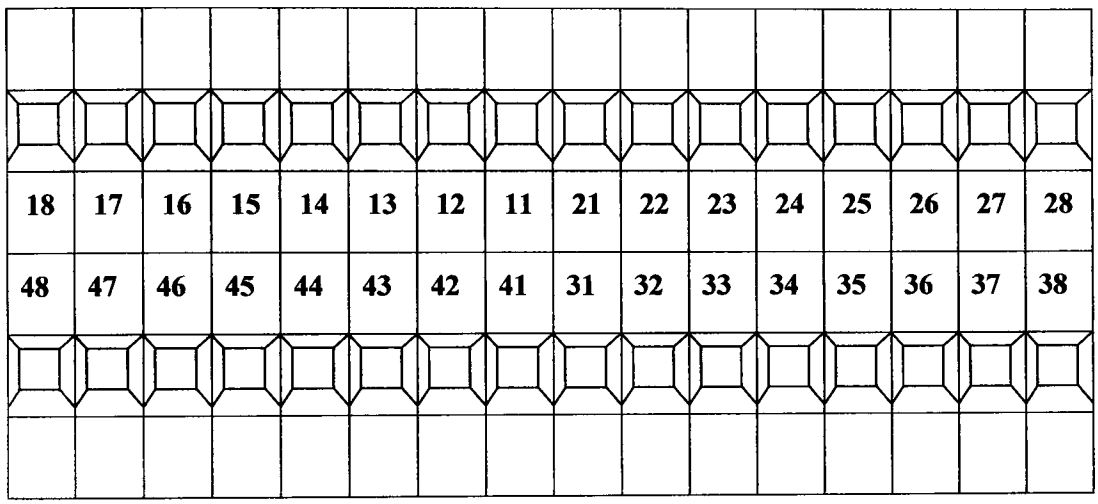
Investigator's signature:..... Date_ _ / _ _ / _ _ _ _

Subject code:	Randomisation no.:

Dental Examination

DMFT score

Right ***Left***



Total DMFT:

Number of natural teeth:

Note: Subject must have at least 18 natural teeth to be eligible for inclusion.

Oral Cavity Examination

	Normal	Abnormal	Describe abnormality
Soft tissues:	☐	☐	

Salivary Flow Rate

Salivary Flow Rate:

1- Unstimulated: __. __ __ ml/min.

2-Stimulated: __. __ __ ml/min.

Note: Unstimulated Salivary Flow Rate must be ≥0.2 ml/min.

Stimulated Salivary Flow Rate must be ≥0.8 ml/min.

Investigator's signature:..... Date_ _ / _ _ / _ _ _ _

Subject code:	Randomisation no.:

Inclusion Criteria Sheet*

	Yes	No
1. Age: Aged between 18-65 years.	☐	☐
2. General Health: Satisfactory medical history with no clinically significant and relevant abnormalities of medical history.	☐	☐
3. Dental Examination: i. In possession of at least 18 natural teeth.	☐	☐
ii. Free from visual signs of untreated caries or periodontal disease.	☐	☐
iii. Not taking any drugs or on special diet hat could affect the salivary flow rate or oral pH.	☐	☐
iv. Unstimulated Salivary flow rate ≥ 0.2 ml/min and Stimulated Salivary flow rate ≥ 0.8 ml/min.	☐	☐
4. Compliance: Understand and is willing, able and likely to comply with all study procedures and restrictions.	☐	☐
5. Consent: Demonstrate understanding of the study and willingness to participate as evidenced by voluntary written informed consent.	☐	☐

*_Note:_ If any of the above questions are answered "No", the subject should be discontinued from the study as a "Screen failure" on the study conclusion page.

Investigator's signature:..... Date_ _ / _ _ / _ _ _ _

Subject code:	Randomisation no.:

Exclusion Criteria Sheet*

	Yes	No
1. Disease:		
a. Current or previous history of serious, severe or unstable physical or psychiatric illness, any medical disorder that may require treatment or make or make the subject unlikely to fully complete the study, or any condition that present undue risk from the study product or procedure (e.g. diabetes, history of aphthous ulcer).	☐	☐
b. A condition or medical history that requires prophylactic antibiotic therapy for dental treatment likely to cause bacteraemia.	☐	☐
2. Medication:		
c. Antimicrobial therapy within 14 days prior to screening.	☐	☐
d. Treatment with antibiotics within 28 days prior to screening.	☐	☐
e. The use of any medication or erosive products that might result in reduced salivary flow rate.	☐	☐
3. Dental Details:		
v. Dental disease that require immediate treatment	☐	☐
vi. Oral surgery or extraction within 6 weeks prior to study initiation.	☐	☐
vii. The wearing of removable prostheses or fixed or removable orthodontic appliances that could affect the conduct of the study.	☐	☐
4. Allergy/Intolerance: Known or suspected intolerance or hypersensitivity to any of the agents used in the study.	☐	☐

Note: If any of the above questions are answered "Yes", the subject should be discontinued from the study as a "Screen failure" on the study conclusion page.

Investigator's signature:..... Date__/__/____

Subject code:	Randomisation no.:

Exclusion Criteria Sheet*

	Yes	No
5. Clinical Trials: Participation in another clinical study or receipt of an investigational drug within 30 days of the screening visit at the start of the study.	☐	☐
6. Employees:		
i. Employees of the study site who were involved in any aspect of the study.	☐	☐
ii. Employees of the sponsor company.	☐	☐

Note: If any of the above questions are answered "Yes", the subject should be discontinued from the study as a "Screen failure" on the study conclusion page.

Investigator's signature:..... Date__/__/____

Leeds Dental Institute

University of Leeds School of Dentistry with the

Dental Hospital at Leeds

Subject code:	**Randomisation no.:**

Fitness and Eligibility to Participate in the Study

In the investigator's opinion, on the basis of the screening assessments and Inclusion and Exclusion criteria, is the subject eligible to participate in the next part of the study?
Yes ☐ No ☐

Investigator's signature:..... Date_ _ / _ _ / _ _ _

Subject code:	Randomisation no.:

Health Check

Note: If the subject experienced any adverse events, please complete the Adverse Events page.

Impressions

	Yes	No
Impressions for Upper arch and lower arch: 1. Are they intact without any distortion?*	☐	☐
2. Do you need to consider any adjustment in construction of the device?*	☐	☐
3. Have you completed the lab's form?	☐	☐
If yes, please specify in the comments space below.		

Comments

Investigator's signature:..... Date_ _ / _ _ / _ _ _ _

Subject code:	Randomisation no.:

Screening Visit Check List

Personnel sheet completed	**Yes** ☐	**No** ☐
Medical history checked	**Yes** ☐	**No** ☐
Dental Examination completed	**Yes** ☐	**No** ☐
Inclusion criteria sheet completed	**Yes** ☐	**No** ☐
Exclusion criteria sheet completed	**Yes** ☐	**No** ☐
Eligibility sheet completed	**Yes** ☐	**No** ☐
Impressions	**Yes** ☐	**No** ☐
Consent given	**Yes** ☐	**No** ☐

Investigator's signature:..... Date_ _/ _ _/ _ _ _ _

Subject code:	Randomisation no.:

Health Check

Note: If the subject experienced any adverse events, please complete the Adverse Events page.
Note: If there are any changes to the subject's medication record, please complete the Concomitant Medications page.

Subject eligibility

	Yes	No
Have there been any deviations from the protocol since the last visit?*	☐	☐
Is the subject still eligible to continue the study?	☐	☐
Has the oral soft tissue check has been conducted?	☐	☐

**If no*, please specify in the comments' space below.

Device fitting (1st month)

	Yes	No
1. Have the device been fitted?*	☐	☐
2. Have the instruction of the protocol given?*	☐	☐

If no, please specify in the comments space.

Wash-out products

	Yes	No
1. Has the wash-out products been given?	☐	☐
2. Has the supervised brushing been done appropriately?	☐	☐
3. Has been the subject randomised? What randomisation number?	☐	☐

Comments

Investigator's signature:..... Date__/__/____

Subject code:	Randomisation no.:

Health Check

Note: If the subject experienced any adverse events, please complete the Adverse Events page.
Note: If there are any changes to the subject's medication record, please complete the Concomitant Medications page.

Subject eligibility

	Yes	No
Have there been any deviations from the protocol since the last visit?*	☐	☐
Is the subject still eligible to continue the study?	☐	☐
Oral Soft tissue check	☐	☐

**If yes*, please specify in the comments' space below.

Appliance Check

	Yes	No	Notes
1. Is the appliance causing problems?	☐	☐	
2. Does the appliance need adjustment?	☐	☐	
3. Has the supervised brushing been reviewed?	☐	☐	

Additional Comments

Investigator's signature:..... Date__/__/____

Subject code:	Randomisation no.:

Health Check

Note: If the subject experienced any adverse events, please complete the Adverse Events page.
Note: If there are any changes to the subject's medication record, please complete the Concomitant Medications page.

Subject eligibility

	Yes	No
Have there been any deviations from the protocol since the last visit?*	☐	☐
Is the subject still eligible to continue the study?	☐	☐
Has the oral soft tissue check has been conducted?	☐	☐

**If no*, please specify in the comments' space below.

Supplies (1st month – 1st visit)

	Yes	No	Amount
4. Slabs fitting.	☐	☐	_____
5. Test Toothpaste.	☐	☐	_____
6. 0.3% Citric Acid bottles (pre-weighed).	☐	☐	_____
1. Water	☐	☐	_____
7. Beakers (predefined levels).	☐	☐	_____
8. Timer.	☐	☐	_____
9. Dipping diary sheet given with instruction.	☐	☐	_____
10. Collect Wash-out products	☐	☐	_____

Toothpaste code

☐

Comments

Investigator's signature:..... Date__/__/____

Subject code:	Randomisation no.:

Health Check

Note: If the subject experienced any adverse events, please complete the Adverse Events page.
Note: If there are any changes to the subject's medication record, please complete the Concomitant Medications page.

Subject eligibility

	Yes	No
Have there been any deviations from the protocol since the last visit?*	☐	☐
Is the subject still eligible to continue the study?	☐	☐
Has the oral soft tissue check has been conducted?	☐	☐

**If no*, please specify in the comments' space below.

Supplies (1st month – 2nd visit)

	Yes	No	Amount
11. 0.3% Citric Acid.	☐	☐	_____
12. Water.	☐	☐	_____

Toothpaste code

☐

Comments

Investigator's signature:..... Date_ _/_ _/_ _ _ _

Subject code:	Randomisation no.:

Health Check

Note: If the subject experienced any adverse events, please complete the Adverse Events page.
Note: If there are any changes to the subject's medication record, please complete the Concomitant Medications page.

Subject eligibility

	Yes	No
Have there been any deviations from the protocol since the last visit?*	☐	☐
Is the subject still eligible to continue the study?	☐	☐
Has the oral soft tissue check has been conducted?	☐	☐

**If no*, please specify in the comments' space below.

Supplies (1st month – 3rd visit)

	Yes	No	Amount
13. 0.3% Citric Acid.	☐	☐	_____
14. Water.	☐	☐	_____

Toothpaste code

☐

Comments

Investigator's signature:..... Date_ _ / _ _ / _ _ _ _

Subject code:	Randomisation no.:

Health Check

Note: If the subject experienced any adverse events, please complete the Adverse Events page.
Note: If there are any changes to the subject's medication record, please complete the Concomitant Medications page.

Subject eligibility

	Yes	No
Have there been any deviations from the protocol since the last visit?*	☐	☐
Is the subject still eligible to continue the study?	☐	☐
Has the oral soft tissue check has been conducted?	☐	☐

**If no*, please specify in the comments' space below.

Collections (1st month – 4th visit)

	Yes	No
15. Has the dental device been collected?	☐	☐
16. Are the slabs intact?	☐	☐
17. Have the slabs been removed from the dental device and immersed in DDW and thymol solution?	☐	☐
18. Have you collected the dipping diary sheet and other products?	☐	☐

Comments

Investigator's signature:..... Date__/__/____

Slab Assessment

Subject code:	Randomisation no.:

Leg No.

Surface Profilometery (SP)

21 Days (Slab no)

Measurement	Initial reading	21 Days reading
Average Lesion Depth		
Date		

Comments:

Investigator's signature:..... Date_ _ / _ _ / _ _ _ _

Slab Assessment

Subject code:	Randomisation no.:

Leg No.

Microhardness (For inclusion)

21 Days (Slab no)

Indent	Initial Length (μm)
1	
2	
3	
4	
5	
Mean	
Date	

Comments:

Investigator's signature:..... Date_ _ / _ _ / _ _ _ _

Leeds Dental Institute

Subject code:	Randomisation no.:

University of Leeds School of Dentistry with the

Dental Hospital at Leeds

Adverse Events

Adverse Event	Onset Date	End Date	Duration		Outcome	Pattern	Intensity	Relation to study	Action taken	Serious*
	__ __ / __ __ / __	__ __ / __ __ / __								
	__ __ / __ __ / __	__ __ / __ __ / __								
	__ __ / __ __ / __	__ __ / __ __ / __								
	__ __ / __ __ / __	__ __ / __ __ / __								
	__ __ / __ __ / __	__ __ / __ __ / __								
	__ __ / __ __ / __	__ __ / __ __ / __								

Duration(Units)	**Outcome**	**Pattern**	**Intensity**	**Relationship to study**	**Action Taken (regarding the study)**	**Serious**
1. S-Seconds 2. M-Minutes 3. H-Hours 4. D-Days	1. Resolved 2. Ongoing	1. Continuous 2. Intermittent	1. Mild 2. Moderate 3. Severe	1. Not relate 2. Unlikely 3. Possible 4. Highly possible	1. None 2. Interrupted 3. Discontinued	1. No 2. Yes*

* All serious adverse events must be reported to the study monitor within 24 hours and require special action

Subject code:	Randomisation no.:

Study Conclusion

Did the subject complete the entire study? Yes ☐ No* ☐

If "No" is checked, please complete the following (please check as an appropriate):

Screen Failure ☐

Adverse Event ☐

Lost of Follow-up ☐

Protocol Deviation ☐ _____

Withdrawal of Volunteer ☐ _____

Other ☐ _____

_____

_____

Investigator's Signature

I confirm that I have reviewed all the data collected in this Case Report Form and take responsibility that the information is accurate and complete.

Study Investigator's Name.....

Study Investigator's Signature.....

Date __ __ / __ __ / __ __ __ __

Appendix 44: dipping diary

Leeds Dental Institute

University of Leeds School of Dentistry with the

Dental Hospital at Leeds

Case Record Form

Study no: 07/H1307/136

The Effect of Fluoridated Toothpastes on Surface Loss of the Dental Hard Tissues under Erosive Condition

DIPPING DIARY LOG

&

APPOINTMENT CARD

SUBJECT CODE ☐☐☐☐☐

RANDOMISATION NUMBER ☐☐☐☐☐

Study Investigator/Dentist

Dr Ahmed Abdullah

Division of Child Dental Health

Principal Investigators

Professor M. S. Duggal

Professor K. J. Toumba

Division of Child Dental Health

STUDY: The Effect of Fluoridated Toothpastes on **Surface loss of the dental hard tissues under erosive conditions**	INFORMATION SHEET

BRIEF INFORMATION FOR COMPLETION OF THE FOLLOWING DIARY CARDS

- Brushing should occur initially in the morning and before bed.
- Guidelines for the suggested frequency of dipping in citric acid:

Frequency	**Suggested Dipping Times**					**Interval**
5 x per day	09.00	12.00	15.00	18.00	21.00	3 hrs

- Each dipping should last for 2 minutes; however the exact length of dipping and the time it takes place should be recorded in the relevant diary log.

Thank you for your co-operation.

Subject code:	Randomisation no.:	Leg No.

Dipping Diary Sheet (1st month)*

1. **Fluoride-free toothpaste's start date.** __ __/__ __/__ __ __ __
2. **Dental device using start date.** __ __/__ __/__ __ __ __
3. **Dental device dipping start date.** __ __/__ __/__ __ __ __

Date	Morning Swishing After Brushing**	Dipping in clear solution***					Evening Swishing After Brushing**
		1st time	2nd time	3rd time	4th time	5th time	

Note:* **Please write the time and duration for each dipping time

** **Brushing is for 1 minute with device is out of mouth followed by 1 minute swishing while the device in the mouth.**

*** **Dipping is for 2 minutes.**

Subject code:	Randomisation no.:	Leg No.

Dipping Diary Sheet 2nd Month*

4. **Fluoride-free toothpaste's start date.** __ __/__ __/__ __ __ __
5. **Dental device using start date.** __ __/__ __/__ __ __ __
6. **Dental device dipping start date.** __ __/__ __/__ __ __ __

Date	Morning Swishing After Brushing**	Dipping in clear solution***					Evening Swishing After Brushing**
		1st time	2nd time	3rd time	4th time	5th time	

Note:* **Please write the time and duration for each dipping time

** **Brushing is for 1 minute with device is out of mouth followed by 1 minute swishing while the device in the mouth.**

*** **Dipping is for 2 minutes.**

Subject code:	Randomisation no.:

Appointments Cards

Day	Date	Cause of visit

STUDY CONCLUSION

Did the subject complete the entire log? Yes ☐ No* ☐

If "No" is checked, please comment on missing dips/ logs and why:

Missed individual dips

Missed entire legs

Other comments

I confirm that I have reviewed all the data collected in this Dipping Diary Log and take responsibility that the information provided by the subject complete.

Signed by (Investigator).....

Print Name (Investigator).....

Dated (DD/MM/YY) ☐☐ ☐☐ ☐☐☐☐

Appendix 45: Age of randomised population (years)

Group	Variable	N	Mean	SD	max	min
Fluoride/Placebo	age	20	35.30	10.00	55.00	22.00
Placebo/Fluoride	age	20	33.00	9.06	58.00	20.00
Total	age	40	34.30	9.47	58.00	20.00

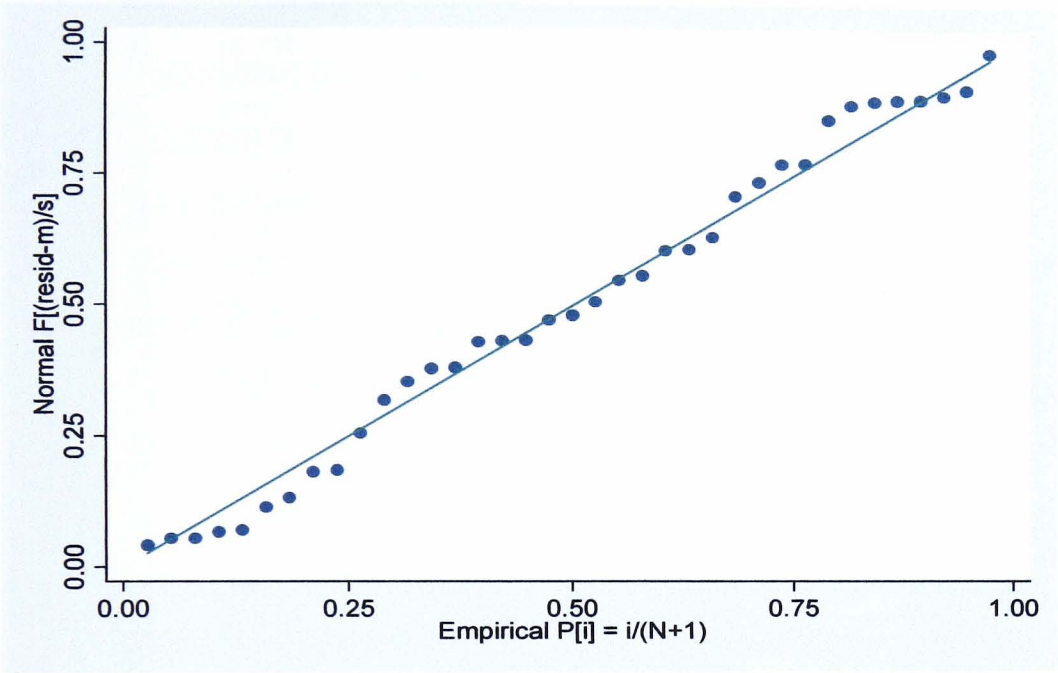
Appendix 46: Gender of randomised population

Group	Sex		Total
	Male	Female	
Fluoride/Placebo	9	11	20
	45.00	55.00	100.00
Placebo/Fluoride	3	17	20
	15.00	85.00	100.00
Total	12	28	40
	30.00	70..00	100.00

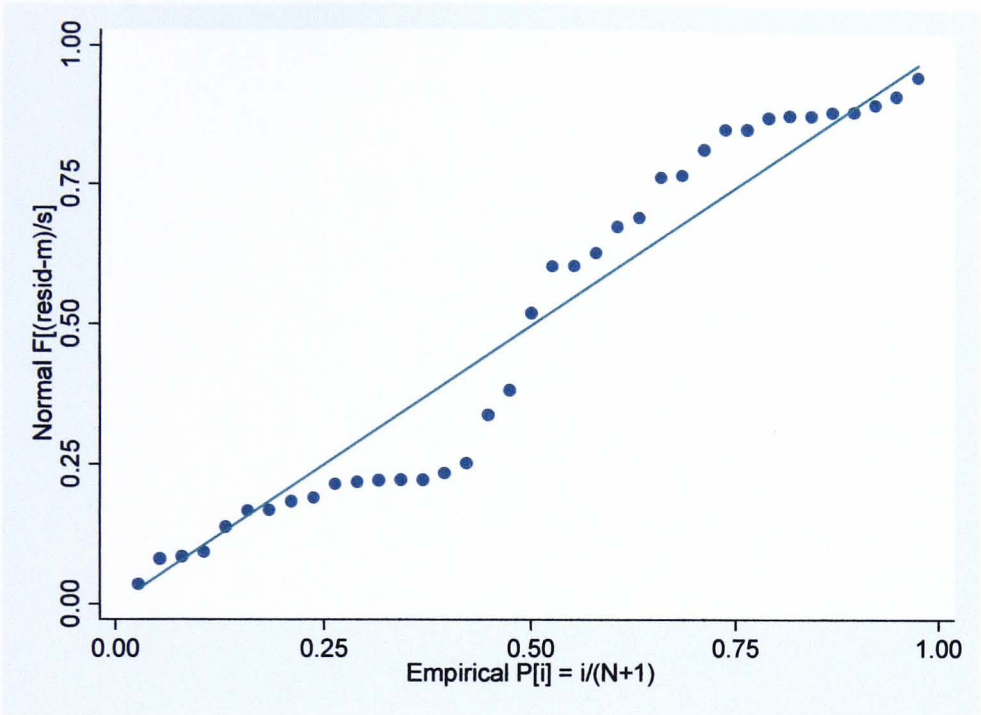
Appendix 47: Enamel surface loss from baseline to Day 21 (Intention to Treat Population).

Subject No.	Leg 1		Leg 2	
	slab 1	Slab 2	Slab 3	Slab 4
1	7.474	9.556	15.417	16.357
2	13.761	12.309	4.545	4.857
3	1.419	2.234	11.734	16.280
4	11.545	15.783	8.329	3.942
5	17.895	13.658	12.76	16.135
6	12.254	11.356	3.336	2.320
7	42.270	57.344	10.112	7.385
8	5.355	3.419	28.633	28.729
9	3.333	2.984	14.263	13.320
10	27.258	30.062	5.795	7.923
11	2.058	2.620	1.786	1.653
12	7.967	9.097	29.353	25.873
13	87.499	77.763	82.869	80.371
14	3.745	5.678	2.782	1.829
15				
16	14.519	13.253	28.431	25.839
17	67.893	81.280	44.314	48.938
18	12.633	15.799	23.896	31.452
19	5.649	2.576	27.553	30.005
20	7.503	3.751	2.341	2.694
21	7.896	3.009	5.631	7.391
22	19.604	28.971	124.061	120.362
23	14.319	29.987	2.525	1.380
24				
25	63.763	44.207	21.828	23.249
26	12.167	17.926	16.149	14.162
27	19.705	18.459	20.462	17.448
28	22.651	19.127	6.120	4.671
29	17.776	21.128	49.421	44.756
30	8.351	5.850	7.963	7.522
31	7.101	8.359	2.820	2.845
32	6.246	5.130	15.836	18.675
33	18.306	16.349	30.226	25.453
34	27.470	20.135	10.849	12.556
35	4.607	8.293	37.175	31.820
36	4.355	4.826	1.828	1.781
37	11.675	12.596	2.849	7.999
38	7.784	8.426	6.030	9.564
39	2.259	3.142	3.528	2.577
40				

Appendix 48: Normal Plot of residuals from ANOVA of Enamel surface loss (Natural log µm) at day 21



Appendix 49: Dentine surface loss from baseline to Day 22 (Intention to Treat Population).

Subject No.	Leg 1		Leg 2	
	slab 1	Slab 2	Slab 3	Slab 4
1	25.800	26.348	33.204	39.823
2	16.294	21.393	2.635	2.556
3	7.852	3.285	27.665	36.192
4	9.925	7.943	4.052	1.420
5	19.102	14.428	16.451	17.521
6	23.246	27.347	2.140	7.465
7	51.000	68.714	22.998	18.762
8	7.145	2.932	26.108	32.769
9	2.462	5.789	18.164	13.598
10	28.793	24.361	7.935	9.834
11	3.976	2.998	2.113	2.447
12	16.093	11.805	59.557	62.563
13	73.511	85.478	76.362	84.214
14	8.929	3.651	4.530	2.410
15				
16	22.450	13.525	41.161	37.937
17	116.484	102.457	71.818	77.171
18	17.005	28.728	28.573	34.968
19	4.914	5.112	44.958	32.621
20	20.669	22.413	15.373	14.724
21	3.504	4.251	3.742	4.160
22	59.719	37.806	139.681	131.854
23	35.671	26.843	5.791	7.130
24				
25	56.990	48.942	37.398	43.766
26	21.649	22.279	24.959	20.572
27	26.289	27.455	27.753	28.868
28	35.558	46.059	5.549	7.866
29	13.847	25.824	67.331	84.390
30	6.569	5.793	4.474	6.287
31	25.075	21.572	6.734	3.895
32	6.408	8.712	30.451	35.583
33	21.612	22.398	29.915	41.522
34	24.879	17.466	15.831	13.738
35	10.598	15.138	47.149	52.750
36	4.291	3.991	3.695	2.557
37	13.312	12.059	3.569	5.270
38	6.637	7.594	8.849	7.125
39	2.694	2.670	2.006	3.145
40				

Appendix 50: Normal Plot of residuals from ANOVA of Dentine surface loss (Natural log µm) at day 21.