Novel Assessments
of Early Enamel Erosion

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ABSTRACT

Dental erosion has been defined as a chemical process that involves the dissolution of enamel and dentine by acid(s) not derived from bacteria when the surrounding aqueous phase is under-saturated with tooth mineral. Clinically however, dental erosion hardly ever occurs exclusively from the other modes of tooth wear such as abrasion and attrition. Hence the term erosive wear has been proposed and used to describe erosion-facilitated wear.

With the prevalence of erosive wear being reported to be on the rise among children and adolescents in many countries, a plethora of oral health products such as dentifrices and mouth rinses had been put forward with claims of increasing the resistance of the enamel and / or dentine from being demineralised. However, the efficacy of these oral health products is still widely questionable as the studies carried out were mainly in vitro and / or in situ studies and the contradictory results were reported by different groups of researchers.

The above mentioned discrepancies are often due to non-standardised in vitro and in situ study designs of erosion studies. Parameters such as type of acid used, concentration of acid used, duration of acid exposure, inclusion or exclusion of abrasion in the study model, ex vivo or in vivo acid challenge, ex vivo or in vivo treatment with oral health product and the nature of pellicle on the specimen are often varied.

The availability of an detection tool which could be used to measure demineralisation on natural tooth surface in vivo would exclude many of the inevitable variability in in situ and in vitro study design, such as the simulation of the intra oral conditions in which the erosion challenge and intervention take place and the nature of the pellicle formed on the specimens. Hence with an in vivo detection tool, it is hopeful that the efficacy of any interventions would be evaluated more accurately and its results expounded to a wider
context. It would be useful if the tool was sensitive to the very early stages of the erosion process as this would entail shorter and more economical study designs.

Arising from the problems discussed above, potential non-invasive assessment methods that could be used clinically to measure demineralisation were explored and it was found that two optical methods, Optical Coherence Tomography (OCT) and Quantitative Light-induced Fluorescence (QLF) were potential methods for the tasks.

The studies described in this thesis were divided into three main big sections; the first being the in vitro validation work of the two optical methods (Chapter 5 and 6), the second, validation of these methods in situ (Chapter 7) and finally the assessment of the efficacy of a high fluoride dentifrice on early enamel erosion using these two methods (Chapter 8).

In the in vitro validation (Chapter 5), it was demonstrated that both QLF and OCT were able to detect erosion-interval related changes in natural surface samples eroded with orange juice for 60 minutes. However, results of Multiple Linear Regression and Paired t test suggest that QLF was more sensitive than OCT in the detection of demineralisation changes in this particular experimental setting. QLF demonstrated a \( R^2 \) value of 0.590 while the best of the OCT outcome measure demonstrated a \( R^2 \) value of 0.319.

Further in vitro study (Chapter 6) was performed to explore the use of a moistened-exposed surface as a reference method for both OCT and QLF as it was felt that it would be beneficial if a coated reference area which posts as an additional step for error could be done away with. Results of this study showed that the moistened-exposed surface could be used a reference method for QLF only but not for OCT.

Furnished with the findings of the above mentioned in vitro validation studies, an in situ validation of both the optical methods was performed (Chapter 7). It was found that OCT and QLF were able to longitudinally measure in situ demineralisation on polished
and natural surfaced enamel which were subjected to 150 minutes of *in vivo* exposure to orange juice. Similar to the results of the *in vitro* study, QLF was found to be more sensitive than OCT in the detection of demineralisation changes on natural surfaced enamel.

The last in situ study (Chapter 8) was to evaluate whether there was a protective effect of treating human enamel with a high-concentrated fluoride dentifrice during an active erosion phase and whether OCT and QLF were able to detect the protective effect. It was found that treating natural surface enamel with a 5000ppm NaF dentifrice increased its resistance against concurrent *in vivo* erosive challenge with an orange juice challenge. QLF was able to detect the protective effect of the 5000ppm NaF dentifrice on natural surface enamel against early *in vivo* erosion with an orange juice challenge regime while OCT did not.
CHAPTER 1

INTRODUCTION
1.1 The Erosion Process

Dental erosion is defined as a chemical process that involves the dissolution of enamel and dentine by acid(s) not derived from bacteria when the surrounding aqueous phase is under saturated with tooth mineral [Larsen, 1990]. Although it is listed in the International Classification of Diseases [WHO, 2007], erosive tissue loss cannot be regarded as a disease per se because erosive and physical wear contribute to the physiological loss of tooth tissue throughout a life time. The erosive acids may be of intrinsic or extrinsic origin. Intrinsic acid is acid regurgitated from the stomach and extrinsic acids are from drinks and food, for example soft drinks, citrus fruit juices and pickles or from industrial source like acidic vapour in a battery factory [Amin et al., 2001; Suyama et al., 2010].

Unlike caries, where it has been established that the destructive effects are both on the surface and within the subsurface region, dental erosion is often described as a solely surface phenomenon. After diffusing through the acquired pellicle, a bacteria free biofilm covering the oral hard and soft tissue, the acid comes in contact with enamel and causes dissolution of the hydroxyapatite crystals. Muerman et al [Meurman et al., 1991] and Meurman and Frank [Meurman and Frank, 1991] investigated enamel erosion by immersing bovine and human enamel specimens in acids for various times. The authors reported that erosion starts within the prism sheath area and then spreads to the prism core, leaving the honey comb appearance of the interprismatic areas when viewed microscopically. Eventually, fresh and unionised acid will then diffuse into the interprismatic areas of enamel and dissolve further mineral in the subsurface region [Eisenburger et al., 2001; Lussi and Hellwig, 2001]. This dissolution process extends a few micrometers below the surface and has been known as softening [Koulourides, 1968]. With time, as softening progresses further into the enamel, dissolution in the most superficial enamel will reach the point where this layer of enamel is lost completely [Eisenburger et al., 2000]. This outflow of ions will subsequently cause a
local pH rise in the tooth substance and in the liquid surface layer in close proximity to the enamel surface [Lussi and Hellwig, 2001]. If no new acid is provided at this point, the erosive process is halted.

However, dental erosion, like dental caries, is a multifactorial process. It is a result of the interplay between several chemical, biological and behavioural factors. The various chemical properties of the extrinsic acid, like its pH, titratable acidity or buffering capacity and calcium-chelation ability determines its erosive potential. The various characteristics of one’s saliva and acquired pellicle have also been found to be important biological factors in the development of dental erosion. Behaviours like the frequency and timing of consuming acidic beverages and food and peculiar eating and drinking habits have been attributed to the development of dental erosion.

1.2 Factors involved in the erosion process

1.2.1 Chemical Factors

The demineralisation of hydroxyapatite crystals can occur either by the attack on the hydroxyapatite crystals by hydrogen ion in acids or by the binding of anions with calcium in the crystals by chelating agents. The effect of the direct attack of hydrogen ion is its combination with the carbonate and/or phosphate compound in the crystal, releasing all of the ions from that region of the crystal surface, leading to direct surface etching. In addition to this effect, some acid ion complexes with calcium, thereby removing it from the hydroxyapatite crystal [Lussi and Jaeggi, 2006].

Several in vitro and in situ studies on humans and animals all show that the erosive potential of different foodstuff and beverages is not exclusively dependent on its pH value, but is also strongly influenced by its calcium, phosphate and to a lesser extent fluoride content, its titratable acidity or buffering capacity and its calcium-chelation properties [Meurman et al., 1990].
The calcium and phosphate contents of a foodstuff or beverage are important determinants of its erosive potential, as they influence the concentration gradients with the local environment of the tooth surface. A low degree of saturation leads to initial surface demineralisation which is followed by a local rise in pH and increased mineral concentration of ions in the liquid surface layer adjacent to the tooth surface. As the dissolution progresses, this liquid surface layer will then become saturated with Ca\(^{2+}\) and P\(_{4}\)\(^{-}\) at which stage the enamel will not demineralise further [Lussi et al., 2011]. However, this saturated surface layer is readily renewed in vivo as new sips of drink are taken or swishing of the drink occurs. Orange juice with a pH of 4 supplemented with calcium and phosphate was found not to erode enamel after immersion in it for 7 days [Larsen and Nyvad, 1999]. Only a small change in the degree of calcium saturation, without any change in its pH, was found to effectively reduce its erosive potential [Barbour et al., 2005]. Attin et al showed that when 1% citric acid was supplemented with different concentrations of calcium and phosphate reduced its erosive potential was reduced [Attin et al., 2003]. They also showed that the most effective reduction of enamel dissolution was achieved by adding either 1.0 mmol/l calcium or a combination of 0.5 mmol/l calcium, 0.5 mmol/l phosphate and 0.5 mmol/l fluoride to citric acid [Attin et al., 2005].

The chelating acids that are of concern in the oral cavity are weak acids like citric acid. The strength of calcium complexion of different acid anions is dependent on the structure of its molecule. Besides directly softening and dissolving dental hard tissue, chelating agents like citric acid can enhance the erosive process in vivo by also interacting with calcium in saliva. It has been shown that up to 32% of the calcium in saliva can be complexed by citrate at concentrations common in fruit juices, thus reducing the super-saturation of saliva and increasing the driving force for dissolution of tooth minerals [Meurman and ten Cate, 1996]. Consequently, acid like citric acid that produces the above described double action is very damaging to dental hard tissues,
while acid like acetic acid that complexes weakly with calcium, plays little part in erosion.

The adhesiveness and displacement of the erosive liquid are other factors to be considered in the erosive process. There appear to be differences in the ability of beverages to adhere to enamel dependant on their thermodynamic properties [Ireland et al., 1995]. The greater the adherence of an acidic substance is, the longer the contact time with the tooth surface and the higher the likelihood of erosion. It has been shown that the displacement of Cola by saliva required 14 mJ/m$^2$, while the displacement of a Cola film required 45 mJ/m$^2$ [Busscher et al., 2000].

In contrast to acidic beverages, there has been little research on the erosive or abrasive potential of acidic food or on the interaction between the two. The cumulative erosive effect of acidic food and beverage could be significant.

In summary, there is no clear-cut critical pH for erosion below which erosion will occur. Even at a low pH, it is possible that other factors are strong enough to prevent erosion, while at a higher pH, it is possible that chemicals that complex calcium can cause erosion. The influence of all the factors described above in the fluid layer immediately in contact with the tooth surface determines whether erosion can proceed.

1.2.2 Biological Factors

1.2.2.1 Saliva

Saliva has been considered the most important biological factor influencing the prevention of dental erosion due to its ability to dilute, clear away and neutralise the erosive agent, form the acquired pellicle and enhance remineralisation of tooth structure. The impact of erosion in patients suffering from salivary flow impairment can clearly demonstrate the importance of saliva in the prevention of dental erosion. Studies have shown that erosion is strongly associated with low salivary flow rate [Jarvinen et al., 1991] and buffering capacity [Lussi and Schaffner, 2000]. In an in vitr...
study, Wetton et al showed that whole saliva from different donors provided different levels of protection to human enamel and dentine against erosion [Wetton et al., 2007]. Rios et al showed that salivary stimulation after an erosive or erosive/abrasive attack reduces the percent change in microhardness of enamel [Rios et al., 2006]. Nevertheless, it is still not known if whole saliva is protective or whether resting or stimulated saliva is more important.

Milosevic and Dawson reported that in vomiting and non-vomiting bulimics bicarbonate levels and salivary viscosity were significantly different to a matched control group [Milosevic and Dawson, 1996]. The findings of this study were also supported by [Rytomaa et al., 1998]. Gadmundsson et al found in their study that the buffering capacity of saliva in 62 patients with erosion was statistically different to a control group [Gudmundsson et al., 1995]. However, Bartlett et al found no salivary differences between adolescents with or without tooth wear [Bartlett et al., 1998] nor did Muerman et al in a study of 117 patients with erosion and reflux disease [Meurman et al., 1994]. These differences might reflect the varying nature of saliva and its collection and measurement techniques. There is a need for further investigations on the relationship of saliva and dental erosion.

1.2.2.2 Pellicle

The salivary acquired pellicle is a protein-based layer which is rapidly formed on dental surfaces after its removal by tooth brushing with dentifrice, chemical dissolution or prophylaxis. This organic layer becomes detectable on dental surfaces after a few minutes of exposure to the oral environment, as previously shown by electron microscopy analyses [Hannig, 1999]. The acquired pellicle may protect against erosion by acting as a diffusion barrier or a perm-selective membrane preventing the direct contact between the acids and the tooth surface [Hannig and Balz, 2001], hence reducing the dissolution rate of hydroxyapatite [Lendenmann et al., 2000]. Nekrashevyych and Stosser showed that a 24-hour pellicle formed in vitro were able to
totally or partially prevent enamel surface microhardness change resulting from a 1-minute exposure to 0.1% citric acid or 5-minute exposure to 1% citric acid respectively [Nekrashevych and Stosser, 2003]. Amaechi et al also showed that 1-hour acquired pellicle formed in situ could protect enamel against demineralisation from 2 hours of in vitro orange juice exposure [Amaechi et al., 1999b]. Although it seems clear that pellicle can interfere in the demineralisation of the dental surfaces, the composition, thickness and maturation time of the pellicle may significantly influence the protection level against dental erosion. In addition to that, some studies have highlighted that the inhibition of demineralisation offered by the pellicle is not a complete one. Hara et al showed that a 2-hour acquired pellicle formed on enamel surfaces in situ was able to reduce the demineralisation provided by orange juice for up to 10 minutes of acid exposure. However, no measurable protection was detected after 20 and 30 minutes of this acid exposure [Hara et al., 2006]. It can be concluded from these pellicle experiments that the pellicle can partially protect against erosion challenges provided they are not too severe or frequent.

1.2.3 Behavioural Factors

Behaviours that increase the contact time of an erosive agent with teeth are likely to be the main driving force leading to erosion in many individuals. The frequency and method of consumption of carbonated drinks contribute to this. It was found that high consumption of carbonated drinks increased the odds of erosion in 12-year-olds by 252% and was found to be a strong predictor of the amount of erosion found in 14-year-olds [Dugmore and Rock, 2004]. Jendsdottir et al also found that there was a threefold higher risk of having erosion in molars or incisors in young adults (19-22 years old) drinking Coca-Cola three times or more a week than in those consuming less. They also found significantly higher erosion scores on molars in subjects drinking more than 1 litre of carbonated drinks per week. On the other hand, many other studies only found weak associations between dental erosion and dietary habits [Milosevic et al., 2004; Nunn et al., 2003]. While trying to determine the strength of association of
potential risk factors with erosion and tooth wear in 14 years old school children, Milosevic et al found the odds ratio for tooth wear/erosion for fizzy drinks was only 1.32 and sports drinks 1.58 [Milosevic et al., 2004]. These differences in observations could be due to variations in the design and implementation of the questionnaires as well as the statistics and sampling methods employed.

Swishing of the erosive agent in the mouth increases agitation of the erosive agent and hence enhances the dissolution process because the acid solution on the surface of the hard tissue is readily renewed [Edwards et al., 1998; Millward et al., 1997]. It has also been postulated that night-time exposure of erosive agents may be particularly destructive because of the absence of salivary flow. Larsen and Nyvad [Larsen and Nyvad, 1999] tried to simulate speed of fluid flow in the mouth in vitro by using laminar fluid jet on specimen.

Previous attempts have been made to simulate in vivo acid flow in vitro such as characterising the stirring speed of acid, beaker size or volume of acid used for the erosive challenge and using an open-system model to simulate intermittent acid flow [Attin et al., 2003]. Some studies have shown the influence of temperature and liquid flow rate on erosion [Amaechi et al., 1999a; West et al., 2000], both of which could have relevance to erosion in vivo as these variables exist in the drinking habits of individuals. Others [Moss, 1998; ten Cate and Imfeld, 1996] have also shown that swallowing speed may have some influence on the rate of erosion.

A comprehensive knowledge of the different risk and protective factors is a prerequisite to initiate adequate preventive measures. The lack of conclusive evidence that any single one of the above described factors are strongly linked to the outcome of erosion or tooth wear indicates that the interplay between the chemical, biological and behavioural factors needs further elucidation and/or there are as yet unidentified factors involved in dental erosion.
1.3 Pattern of wear

Early diagnosis of dental erosion is important. Dental professionals may overlook the very early stages of dental erosion, dismissing minor tooth surface loss (TSL) as a normal and inevitable occurrence of daily living, being within normal limits and thus not requiring any specific intervention. Only at the later stages of the process where dentine is exposed and the appearance and shape of the teeth are significantly altered does the condition becomes evident at routine examination. There is currently no device available for the specific detection of dental erosion and therefore clinical appearance is currently the most important diagnostic feature.

Some typical signs of early erosion are firstly the appearance of a smooth silky-glazed appearance or loss of surface characteristics, followed by increased incisal translucency, grooving of lower incisal edges (Figure 1.1), cupping on non-contact areas of occlusal surfaces (Figure 1.2) and restorations with margins protruding above adjacent tooth structure. Typically, broad concavities on the labial surfaces of the upper incisors are commonly associated with dietary acids and erosive lesions on the palatal surfaces of upper incisors are more frequently associated with regurgitated stomach juices [Bartlett et al., 1996; Moazzez et al., 2004; Tantbirojn et al., 2012]. The pattern of erosion might be also influenced by salivary flow [Milosevic and Dawson, 1996], pellicle thickness [Duschner et al., 2000] or gingival sulcular flow [Lussi et al., 2004]. The latter may be responsible for the preservation of a thin band of enamel along the gingival margins of worn upper incisors [Lussi et al., 2004]. Interactions between erosion, abrasion and attrition in tooth surface loss

Compounding the multifactorial nature of dental erosion is the fact that it almost always occurs in combination with the other processes of tooth wear, like abrasion and attrition. Both clinical and experimental observations show that individual wear mechanisms rarely act alone but interact with each other, even though one may predominate [Bartlett and Smith, 2000; Meurman and Sorvari, 2000]. In tribological
terms, erosion would be described as a form of tribochemical wear where the attack by a chemical agent weakens the superficial region of the material and enhances its susceptibility to mechanical forces [Mair, 2000]. In vivo, erosion could involve two types of wear of enamel: The direct removal of hard tissue by complete dissolution and the creation of a thin softened layer, which is vulnerable to subsequent mechanical wear [Featherstone and Lussi, 2006].

More case-control or longitudinal studies concentrating on individuals exposed predominantly to dental erosion rather than the other mechanical type of wear could potentially yield more useful information of the clinical presentations of this process and the role of the plethora of risk factors in the development of dental erosion.

1.4 Prevalence and Incidence

There is some evidence that the prevalence of dental erosion is growing in school aged children. In the 2003 United Kingdom National Children’s Dental Health Survey, the proportion of children with dental erosion observed on the palatal surfaces of incisors increased at each examined age group with 14% of 8-year-olds and 33% of 15-year-olds. The proportion of first permanent molars with erosion on the occlusal surface also rose at each age group with 10%, 19% and 22% affected at age 8, 12 and 15 years respectively. In addition to that, a six percentage point increase in the proportion of affected 15-year-olds and a three percentage point increase in the proportion of affected 12-year-olds were reported when compared to a similar 1993 survey [Chadwick et al., 2006]. In the North-West of England, Bardsley et al in 2004 found that 53% of 14-year-olds have at least one tooth surface with exposed dentine [Bardsley et al., 2004]. However, in another survey Dugmore and Rock in 2004 showed a prevalence of erosion of 59.7% in a cohort of 12-year-olds in the United Kingdom [Dugmore and Rock, 2004]. Al-Dlaigan et al, on the other hand found that 48% of 14-year-olds in Birmingham had erosion within enamel [Al-Dlaigan et al., 2001].
Truin et al found that 24% of 12-year-olds in The Hague, the Netherlands exhibited signs of erosion [Truin et al., 2005] and in Reykjavik, Iceland, 21.6% of a cohort sample of 15-year-olds was found to suffer from dental erosion [Arnadottir et al., 2003].

In most of the described surveys, significantly more erosion was detected in boys than girls. The prevalence ranged from 15% to 60% but it is difficult to compare the above data directly mainly due to variations in indices employed, surfaces and tooth that were used for scoring and sampling methods.

Incidence data concerning dental erosion is scarce. Dugmore and Rock in 2003 examined 1,308 children at the age of 12 years and subsequently 2 years later. Approximately 12% of erosion free children at 12 years developed the condition over the following 2 years. New or more advanced lesions were seen in 27% of the children over the study period [Dugmore and Rock, 2003]. In another study on adults, an attempt was made to determine the progression of erosive defects in 55 persons who were examined on two occasions six years apart [Lussi and Schaffner, 2000]. All the subjects were informed about the risk of erosive tooth wear but no active preventive care during the study period was performed. A distinct progression of erosion on occlusal and buccal surfaces was found. The occurrence of occlusal erosions with involvement of dentine rose from 3% to 8% in the 26-30 years old at the first examination and from 8% to 26% in the 46-50 years old at the first examination.

1.5 Indices

As currently there are no established clinical assessment tools available for the detection and monitoring of erosion, its detection is still very much dependant on visual identification. Many different clinical erosion and tooth surface loss indices have been developed and used mostly for epidemiological and clinical purposes.
Most of these indices consist of a combination of quantitative and qualitative criteria and are essentially dichotomised to the presence or absence of dentine. The quantitative criteria used are objective physical measurements like the area of worn surfaces and depth of cuppings. Qualitative criteria used are, for example, descriptive terms like slight, mild, moderate and severe or descriptions of the surface texture of enamel. These are more subjective and need a high level of training and calibration to render them useful.

Eccles originally classified lesions broadly as early, small and advanced, with no strict criteria definitions, thus allowing wide interpretation [Eccles, 1978]. Subsequently, the index was refined and expanded, with greater emphasis on the descriptive criteria [Eccles, 1979]. It not only graded severity of erosion but also site of erosion and is considered as one of the cardinal indices from which others have evolved. Smith and Knight [Smith and Knight, 1984] took Eccles' idea a stage further producing the Tooth Wear Index (TWI) wherein all four visible surfaces of all teeth were scored for wear, irrespective of its aetiology. This was to avoid confusion associated with terminology and translation or differences of opinion for the diagnosis of aetiology based on clinical findings. However, some problems have been identified with TWI, for example the time necessary to score the whole dentition and the comparisons with different threshold levels for different age groups. Donachie and Walls [Donachie and Walls, 1995] suggested a need to increase the sensitivity of TWI at extremes of tooth wear for the elderly while Millward et al [Millward et al., 1994] made adjustments to it for the study of erosion in the primary and young secondary dentitions by excluding cervical surfaces. Linkosalo and Markkanen [Linkosalo and Markkanen, 1985] utilised a qualitative index with listed diagnostic criteria and a four-point scale to grade severity of erosion. Their scoring system was then modified by Lussi et al [Lussi, 1996] to create an erosive index that has been used widely by European researchers to score buccal, lingual and occlusal surfaces of all teeth except the third molars (Table 1.1). Lussi’s index has subsequently been modified to be used in large scale epidemiological studies by van
Rijkom et al [van Rijkom et al., 2002] and Arnadottir et al [Arnadottir et al., 2010; Arnadottir et al., 2003].

The indices described above have been used mainly in epidemiological settings. In clinical situations, Oilo et al [Oilo et al., 1987] pioneered a different type of scoring system, with its criteria based upon treatment need but this index requires considerable experience for reliable use because individuals with differing clinical backgrounds will produce differing results. There have also been attempts to visualise, measure and monitor the amount of worn enamel or exposed dentine indirectly on serial study casts. Fareed et al, Schlueter et al [Fareed et al., 1990; Schlueter et al., 2005] and Larsen et al [Larsen et al., 2000] recommended a new clinical index based on a combination of clinical examination, photographs and study casts with complicated qualitative and quantitative criteria.

Due to the existence of a plethora of indices for tooth wear with different qualifiers and scores for different aetiology it was accepted among researchers of tooth wear and erosion that comparisons of reported incidences and prevalence of erosion was almost impossible [Young et al., 2008]. Hence a workshop dedicated to developing a standardised erosion index was organised and a new scoring system, the Basic Erosive Wear Examination (BEWE), was the outcome of the workshop. It was designed to provide a simple tool for use in general practice and to allow comparison to other more discriminative indices. It is a partial scoring system recording the most severely affected surface in a sextant with a four level score (Table 1.2) and the cumulative score classified and matched to risk levels which guide the management of the condition. The BEWE allows re-analysis and integration of results from existing studies and it was hoped that it would avoid the continued proliferation of indices. The BEWE further aims to increase the awareness of tooth erosion amongst clinicians and general dental practitioners and to provide a guide as to its management [Bartlett et al., 2008].
Most of the descriptions of the above indices did not elaborate the conditions under which the teeth were examined such as whether they should be examined under dry or wet conditions. This factor significantly affects the result or outcome of the scoring, as initial enamel erosion lesions can easily be masked under wet conditions. There is also a lack of illustrations to accompany the descriptions of the scores of each index.

It is doubtful whether any of the indices described above are sensitive enough to monitor all but the most severe changes in dental erosion or tooth wear and these certainly cannot be used to measure the rate of wear. In addition to that, the distinctions between the various levels of scores are generally too crude to be used to monitor the progression of a lesion. The scores for the indices suffer from subjective clinical interpretation and hence it has been suggested that the measurement validity for a purely erosion index is doubtful [Milosevic, 2011]. To date, there is no one ideal index that meets all the requirements of all three purposes of epidemiological study, clinical staging and longitudinal monitoring of the effect of interventions (such as behavioural modification or anti-erosive dental health products) on dental erosion or erosive wear.
1.6 Summary

Although epidemiological studies have reported high prevalence of dental erosive wear, these studies have yet to identify any significant associated biological or behavioural risk factors. The lack of evidence in any associated risk factors could be due a few main reasons:

i. The multifactorial nature of the erosion process makes the results of laboratory studies hard to be projected into clinical situation.

ii. In the mouth, the erosion process does not occur exclusively from the other wear processes such as attrition and abrasion.

iii. Risk factors were most of the time being elicited via cross-sectional studies using questionnaires looking into past or present behaviours. Few longitudinal studies have been performed.

iv. The absence of a sensitive, validated and objective clinical assessment tool and / or index for detecting erosion and / or erosive wear.

The subsequent chapters discuss in more depth first the laboratory assessment tools and subsequently clinical assessment methods and models for enamel erosion.
1.7 References


1.8 Tables

Table 1.1

Index for erosive wear according to Lussi [Lussi, A. 1996].

<table>
<thead>
<tr>
<th>Surface</th>
<th>Score</th>
<th>Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Facial</td>
<td>0</td>
<td>No erosion. Surface with a smooth, silky glazed appearance, possible absence of developmental ridges</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>Loss of surface enamel. Intact enamel cervical to the erosive lesion; concavity on enamel where breadth clearly exceeds depth, thus distinguishing it from toothbrush abrasion. Undulating borders of the lesion are possible and dentine is not involved</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>Involvement of dentine for less than half of tooth surface</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>Involvement of dentine for more than half of tooth surface</td>
</tr>
<tr>
<td>Occlusal or Lingual</td>
<td>0</td>
<td>No erosion. Surface with a smooth, silky glazed appearance, possible absence of developmental ridges</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>Slight erosion, rounded cusps, edges of restorations rising above the level of adjacent tooth surface, grooves on occlusal aspects. Loss of surface enamel. Dentine is not involved</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>Severe erosions, more pronounced signs than in grade 1. Dentine is involved</td>
</tr>
</tbody>
</table>
Table 1.2
The Basic Erosive Wear Examination (BEWE) index. [Bartlett et al., 2008]

<table>
<thead>
<tr>
<th>Score</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>No erosive tooth wear</td>
</tr>
<tr>
<td>1</td>
<td>Initial loss of surface texture</td>
</tr>
<tr>
<td>2*</td>
<td>Distinct defect, hard tissue loss, &lt;50% of the surface area</td>
</tr>
<tr>
<td>3*</td>
<td>Hard tissue loss &gt; 50% of the surface area</td>
</tr>
</tbody>
</table>

*in scores 2 and 3 dentine often is involved
1.9 Figures

Figure 1.1

Figure shows grooves on the incisal edges of lower incisors.
Figure 1.2

Figure shows ‘cupping’ of the cusps on the occlusal surface of a mandibular molar.
CHAPTER 2

METHODS OF ASSESSING EARLY ENAMEL EROSION
2.1 Significance of Assessing Early Enamel Erosion

During the early stage of dental erosion, the initial acid attacks on the tooth surface result in a superficial partial dissolution of mineral often referred to as the surface softening stage [Arends and ten Cate, 1981; Koulourides, 1968] where no surface loss in depth has been observed yet. If the acid challenge persists, this softened layer will eventually be lost either by complete dissolution [Cheng et al., 2009; Eisenburger et al., 2000] or worn away by mechanical forces from abrasion or attrition in vivo.

As surface bulk loss has yet to occur at this stage of erosion, it would seem reasonable to assume that remineralisation of this demineralised enamel is possible. Hence in depth understanding of the various aspects of this stage of erosion is important. The difference in the dissolution process and the resulting ultrastructure in different enamel substrates such as polished or unpolished natural enamel; human or bovine enamel, and how this softened enamel can be modified, reduced or prevented are of clinical significance. The quality, nature and amount of information of this softened layer of enamel will depend on the tools and methods available to assess it.

In the oral cavity, the contact of the teeth with an acidic substrate is usually limited to a few seconds before clearance by saliva. This means that under natural conditions early erosive lesions are created with very small loss of mineral. Detection of these small surface changes would allow reducing the contact of an acidic substrate with the tooth surface in experiments to a time period resembling intra-oral conditions. Feasibility to detect these small alterations would enable one to reduce contact of the substrate with a tooth to a single and short event instead of long or repeated procedures which are disadvantageous especially in in situ and in vivo experiments.

Methods for assessing erosion have previously been reviewed [Attin, 2006; Azzopardi et al., 2000; Barbour and Rees, 2004; Field et al., 2010; Grenby, 1996] and the reviews covered the assessments methods and models for both early and advanced erosion that involved surface loss. The focus of this chapter, however, is on the in vitro
assessment tools available for the assessment of early enamel erosion whilst the in vivo tools and study design for early enamel erosion will be discussed in Chapter 3.

Both quantitative assessments and qualitative assessments are important and complement each other. In order to obtain objective and measurable data, quantitative analyses are preferred. However, with qualitative assessment techniques such as Scanning Electron Microscope (SEM) and Confocal Laser Scanning Microscopy (CLSM), changes of tooth structure can be visualised. These microscopy methods complement the quantitative methods for example by demonstrating ultrastructural impacts of different erosive agents on different enamel substrates (be it polished or natural surface, human or bovine sample).

2.2 Quantitative Assessment

Quantitative assessments of demineralisation can be approached directly via assessment of minerals / ion exchanges during the process of demineralisation or indirectly by correlating the degree of demineralisation with changes in the:

a. Physical properties specifically surface hardness and / or

b. Degree of optical changes in the softened enamel.

Quantitative assessments of early demineralisation can also be evaluated by assessing the extent of demineralisation in the subsurface enamel or, in other words, the thickness of the softened layer.

2.2.1 Non-contact Profilometry with ultrasonication

Profilometry, or surfometry, has been extensively used to characterise enamel loss caused by erosion. The surface loss of enamel and its surface roughness could be determined by scanning with a small metal or diamond contact stylus (with a diameter of 2 - 20 µm) or a non-contact laser beam [Hooper et al., 2004; West et al., 2003]. To assess the effect of an erosive agent, part of the surface is protected with adhesive tape, nail varnish or similar layer and the unprotected surface is exposed to the erosive
agents, thus providing a direct comparison between treated and protected areas. The sample surface is scanned before and after erosion, and the amount of material loss can be measured from the trace produced. In laser profilometry, there is no direct physical contact between the probe and the surface. Hence no damage occurs to the soft eroded surface. Interferometry is used to build up a map of the surface and scans conducted after treatment can be matched with the baseline scan using coordinate software in order to determine differences in height between these two scans [Attin et al., 2005; Venables et al., 2005]. In these cases, it is extremely important to ensure correct repositioning of the sample in the profilometer for the two readings. Since material loss is assessed rather than surface softening, profilometry is more suited for measurement of advanced erosion where surface loss has occurred.

However, Eisenburger et al [Eisenburger et al., 2001] attempted to use contact profilometry together with ultrasonication to assess erosion depths and the extent of surface softening. The softened surface was removed with ultrasonication and the resulting crater was measured with a contact or non-contact profilometer. This has provided an estimate of the thickness of the softened layer of approximately 2–5 µm. This method has since been used to assess the influence of liquid temperature and flow rate on surface softening [Eisenburger and Addy, 2003], the effects of modification of soft drinks on its erosive potential [Hughes et al., 2002] and to compare the effects of tooth brushing relative to ultrasonication.

Profilometry has the advantage of being a simple and fast assessment method over a relatively wide area of enamel. However, similar to indentation techniques, the enamel sample has to be ground flat prior to use.

### 2.2.2 Surface Hardness

Hardness, as measured by penetration, is a measure of the mechanical resilience of enamel. Featherstone et al [Featherstone et al., 1983] and Kielbassa et al [Kielbassa et al., 1999] reported that hardness profiles can be used as a direct measure of mineral
gain or loss as a consequence of either remineralisation or demineralisation. It is one of the most extensively used methods in de- and remineralisation studies, especially with regards to erosion. With hardness measurements, early stages of enamel and dentin dissolution, which are associated with weakening of the surface, can be determined. The basic method involves the indentation of a diamond tip of known geometrical dimensions for a given load and duration. Two techniques are commonly used to measure enamel hardness. Surface hardness, or microindentation, is the more established and traditional method, whereas nanoindentation (also known as ultra-microindentation) is emerging as a new technique that is readily applicable to enamel erosion.

2.2.2.1 Microhardness

The microindentation technique yields data in arbitrary units, usually Knoop hardness number (KHN) or Vickers hardness number (VHN). An important difference between the Knoop and Vickers microhardness techniques is the penetration depth of the diamond indenter. For a given indentation length, the Vickers indenter penetrates 4.5 times deeper than the Knoop indenter [Knoop et al., 1939]. The hardness measured by microindentation is affected not only by the immediately surrounding material, but also by the material at a distance of approximately 10 times the dimensions of the indentation [Tsui and Pharr, 1999]. Thus, microhardness of enamel is a function of its mechanical properties at some tens to hundreds of micrometres from the measured enamel surface. Typically in sound enamel, the depths of the indentations are in micrometres or tens of micrometres [Arends and ten Bosch, 1992].

Microindentation has also been used to compare enamel erosion by different solutions. Statistically significant changes due to erosion by cola drinks, fruit juices, sport drinks, red wine, bleaching agents, citric acid solution and acidic lozenges have been reported. Softening of enamel samples followed by re-hardening has been observed after exposure to saliva, soft cheeses and milk. In some studies, microhardness changes
after short exposure times were observed, for example, five minutes of exposure to a cola drink. In these studies, agitation of the cola was shown to increase the degree of erosion and the presence of an early salivary pellicle was shown to reduce erosion. Other studies using microindentation have also indicated that salivary pellicle reduces the degree of softening caused by erosion.

Advantages: The popularity of this technique is mainly associated with the low initial start up cost and the ease with which data can be acquired. It is faster and simpler to perform than nanohardness measurements.

Disadvantages: The in vitro study of Buchalla et al [Buchalla et al., 2008] does not recommend calculating mineral content data from micro- or nanohardness profiles. Microhardness is determined by measuring the dimensions of the indent left in the enamel surface. As such, only plastic (permanent) deformation is investigated and there is no information regarding the elastic response and recovery of the surface.

There is also the inherent limitation of the necessity to perform the test on flat specimens. Caldwell et al [Caldwell et al., 1957] previously attempted to assess natural curved enamel specimens. At least 10 indentations, usually many more, were placed on each surface under study. The impressions were usually made progressively along a line down the centre of the surface from the cervical margin to the incisal edge. Only impressions symmetrical to the eye were measured and recorded. For some teeth, the hardness, as calculated from the length of the long diagonal of the impression, was corrected to the hardness value corresponding to the actual area of the indentations in mm². This gave a corrected Knoop Number.

A perfect indentation has a ratio of the long to the short axis of 7.11 to 1. The corresponding ratios obtained on intact tooth surfaces were found at times to vary significantly from the theoretical value. The most likely interpretation for this variation is that the surfaces are curved.
2.2.2.2 Nanohardness

Nanohardness not only yields hardness values but also yields the reduced elastic modulus in the SI unit of Pascals (Nm$^2$). Nanoindentations in sound enamel have sub-micrometre indentation depths, typically around 200 nm [Finke et al., 2001] and are only affected by the material to a depth of a few micrometres [Barbour and Rees, 2004].

Nanoinindentation is a comparatively new technique and there are only a few reports of its use to investigate enamel erosion. In the first of such report, and the only one of in situ erosion, enamel samples were placed in an intra-oral appliance in the mouth of a volunteer who drank one of three drinks. Nanoindentation demonstrated clear differences between the mechanical properties of the enamel samples as a function of the different drinks, with orange juice resulting in significantly more softening than mineral water or a blackcurrant drink [Finke et al., 2001]. A related study in which in vitro erosion by soft drinks was investigated using ‘ultra-microindentation’ apparatus also indicated different degrees of softening by different drinks [Mahoney et al., 2003]. In a preliminary study, another group observed changes in the mechanical properties of enamel after storage in deionised water and saline solution, and advised caution in the choice of storage medium for tooth specimens [Habelitz et al., 2002]. More recently, a series of investigations of in vitro enamel erosion has previously demonstrated unknown features of the dependence of enamel erosion on the calcium and phosphate concentrations, degree of saturation and pH of acidic solutions [Barbour et al., 2003a, 2005; Barbour et al., 2003b]. These studies used exposure times of 30 seconds – 10 minutes, which is comparable to the clearance times of acids in the mouth. This demonstrates that nanoindentation is extremely sensitive to changes in the mechanical properties of the enamel surface and is likely to find increasing application in enamel erosion research.
Advantages: With a typical nanoindentation apparatus, the displacement of the tip as a function of the applied load, is continually monitored during indentation, and as such the plastic and elastic deformation of the surface can be determined. In some types of nanoindentation apparatus, the tip can be scanned across the substrate surface line by line, building up an image of the area in contact with the tip. This is particularly useful when analysing inhomogeneous substrates, as different regions of the surface can be identified and indented, as well as in rough substrates, since locally flat areas can be selected for indentation. Since dissolved enamel surfaces are both inhomogeneous and rough, nanoindentation imaging is beneficial when applied to enamel dissolution studies. In practical terms, both the hardness (plastic, permanent deformation) and the elastic modulus (or Young’s modulus) may be readily calculated from nanoindentation load–displacement data. Elastic modulus data may be useful in erosion experiments, since it is has been shown in studies of thin films and of enamel erosion [Barbour et al., 2003a] to be more sensitive than hardness to the presence of underlying hard material (intact enamel in the case of erosion).

Disadvantages: A drawback of both techniques is that the enamel surface must be polished to provide a flat substrate prior to erosion. The outer layer of the enamel, which contains much greater concentrations of fluoride and lower concentrations of magnesium and carbonate than enamel, is thus removed. Since the solubility of inner enamel is known to be higher than that of surface enamel, erosion proceeds more quickly in polished samples.

2.2.3 Microradiography

Microradiography is a technique where a photographic plate [Arends and ten Bosch, 1992] or a photon counter [Anderson et al., 1998] is used to record the penetrating radiation of a beam of X-rays incident on an enamel section. The degree of blackening of the film or the photon density, together with a calibration sample, provides a map of the mineral density of the enamel. Microradiography, predominantly used in the
investigation of caries, has been adopted for detecting erosive mineral loss \textit{in vitro} and has the potential to assess both material loss and the extent of softening. The technique is usually divided into one of three ‘generations’ of microradiography. These are: transverse microradiography (TMR), used for thin sections (50–200\,µm) and radiographed perpendicular to the sample; longitudinal microradiography (LMR), used for thick sections of teeth; and wavelength-independent microradiography, which is used to quantify mineral content in whole teeth [Arends et al., 1997].

\textbf{2.2.3.1 TMR}

TMR, which is the more widely used technique, is a valid tool for quantitative assessment of the mineral content as a function of depth from the surface for caries and caries-like lesions [Arends and ten Bosch, 1992]. It has been adopted for detecting erosive mineral loss \textit{in vitro} and has the potential of assessing both material loss, and the extent of softening. From the in-depth profiles, the lesion depth and mineral loss integrated over the entire depth (\(\Delta Z\)) of the lesion can be calculated. Lesion depth is usually defined up to the point, where the mineral content reaches 95% of the mineral content of sound enamel or dentin. Hall et al. [Hall et al., 1997] found a strong correlation between mineral loss determined by either TMR or profilometry even for discrimination of early erosive lesions caused by erosion times of less than 1 h.

Studies have been carried out to correlate other methods of assessment with mineral loss as determined by TMR. One study demonstrated a strong correlation between microradiography and profilometry of erosive lesions [Hall et al., 1997]. Margolis et al [Margolis et al., 1999] reported that there may be a good correlation between chemical analysis of hydroxyapatite dissolution with microradiography. It has since been used as the gold standard by which new assessment techniques were validated [Amaechi et al., 2003; Elton et al., 2009; Jones et al., 2006; Pretty et al., 2004].
2.2.4 Optical Methods

2.2.4.1 Optical Coherence Tomography (OCT)

Light offers several advantages not found in other imaging techniques. It is innocuous, as it is non-ionising and hence not tissue-damaging. It is also fairly easy to generate and detect. The use of light as a high resolution imaging tool in dental applications has been compromised by the turbid nature of dental tissue. When light travels through turbid media, it undergoes arbitrary changes in propagation directions and loss of coherence due to scattering from the random distribution of inhomogeneous optical scatterers. The quality of the image is severely affected by this scattering. Recent developments in OCT have made it possible to overcome this limitation by using interferometric cross-correlation techniques to detect the coherent backscattered components of short coherent light. A cross sectional image is produced by transversely scanning the beam across the sample and collecting a reflectance versus depth profile at each transverse location. The reflectance intensities are recorded digitally on a grey-scale image as a function of transverse and axial distances.

OCT was pioneered by Fujimoto et al [Fujimoto et al., 1995] for in vivo imaging of the human eye. It is able to facilitate qualitative and quantitative assessments of oral tissue. OCT is able to quantitatively monitor mineral changes in a caries lesion on a longitudinal basis in bovine teeth in vivo. The OCT system used a wavelength of 850 – 1310 nm, resulting in image depths of 0.6 -2.0 mm. Jones et al [Jones et al., 2006] used OCT to successfully evaluate artificial caries severity and depth in human teeth in vitro. As the application of OCT to dentistry is relatively new, there is still much work to be done to assess its full potential. Of clinical relevance is the development of prototype hand pieces for intra-oral OCT. Though there are already several in vitro studies looking at the validity and efficacy of using OCT to evaluate demineralisation, there are few reports of its use in vivo. One of the few in vivo studies that has been
performed using OCT are by Fried et al [Fried et al., 2010] and Wilder-Smith et al [Wilder-Smith et al., 2009]

2.2.4.2 Quantitative Light-Induced Fluorescence (QLF)

Quantitative light induced fluorescence (QLF) is a non destructive diagnostic method for the longitudinal assessment of early caries lesions over time. When a tooth becomes carious the fluorescence radiance at the location of the caries lesion decreases. The fluorescence image of enamel with incipient lesions can be digitised, then the fluorescence loss in the lesion quantified in comparison to the fluorescence radiance level of the surrounding sound enamel. Changes in fluorescence radiance and lesion area can be followed in time to measure lesion behaviour. The amount of fluorescence radiance loss is related to the mineral loss in the lesions. The technique can be used in vitro, in situ and in vivo to monitor mineral changes in lesions [van der Veen and de Josselin de Jong, 2000]. Applications of QLF are found in the testing of products designed to inhibit demineralization and promote remineralisation of caries. The method has been successfully applied to smooth surfaces as well as occlusal surfaces.

Several theories have been suggested to explain the decrease in fluorescence in early demineralised lesion especially incipient caries [Spitzer and ten Bosch, 1975; ten Bosch, 1996] and Angmar-Mansson and ten Bosch provided a schematic overview of the scattering, absorption and fluorescence mechanism in sound and carious enamel with sound dentine underneath [Angmar-Mansson and ten Bosch, 2001]. The penetration depth of light in demineralised lesion is smaller than in sound enamel. Photons entering carious enamel are highly scattered [ten Bosch, 1996]. Most light entering the lesion is volume reflected from the lesion and the chance of photon absorption and fluorescence is small. Photons travel further in sound enamel and along their path may be absorbed by a fluorophore leading to excitation of fluorescent photons. Light entering sound enamel may reach the DEJ and dentine where the
chance of absorption by a fluorophore is a magnitude higher than in enamel. Many more fluorescence photons are emitted from the sound enamel than from the demineralised lesion. The scattering coefficient of a white spot lesion is a factor 5 to 10 higher than that of the surrounding sound enamel [Spitzer and ten Bosch, 1975]. In a white spot lesion the incident light is scattered more often than in sound enamel as a result of the disintegration of the crystalline structure resulting in more internal reflection sites. Increased scattering implies that the 'mean free path of photon transport' inside a lesion is shorter than in sound enamel. The chance for a photon to be absorbed and fluorescence emitted is therefore higher in sound enamel than in the white spot and it is assumed that more fluorescent light is emitted from the sound enamel than from the lesion.

### 2.2.5 Ionic changes

#### 2.2.5.1 Calcium and phosphate ion analysis

Chemical analysis has been used extensively to investigate the kinetics and thermodynamics of enamel and hydroxyapatite dissolution by measuring the concentrations of calcium and phosphate released into the dissolving solution, as well as the pH and uptake or release of minor constituents of enamel such as fluoride or magnesium. Calcium analysis is usually performed using atomic absorption spectroscopy and phosphate concentration is usually determined using spectrophotometry of a coloured phosphate complex [Barbour and Rees, 2004].

The pH stat is a technique frequently applied to studies of chemically pure hydroxyapatite dissolution [Shellis et al., 1993] but is readily applicable to the investigation of real time enamel erosion [Barbour and Rees, 2004]. It is a chemical analysis device, which makes use of the fact that, on dissolution, hydroxyapatite and enamel release hydroxyl (OH\textsuperscript{−}) ions. Thus, by measuring the change in pH of a solution, it is possible to determine the rate of release of OH\textsuperscript{−} ions, and from this the rate of hydroxyapatite or enamel dissolution can be calculated. The pH stat includes a
feedback loop, which acts to hold the pH at a stationary value. For example, enamel dissolution may be investigated in an acidic solution at, say, pH 3.3. When the enamel sample is immersed in the acid, it starts to dissolve, releasing $\text{OH}^-$ ions which neutralise $\text{H}^+$ ions and cause the pH to increase. The pH stat equipment automatically adds acid to compensate for this neutralisation of $\text{H}^+$ ions and maintains the pH at the initial value of 3.3. By measuring the rate at which it is necessary to add acid to maintain a constant pH, it is possible to calculate the dissolution rate of the enamel at a chosen pH.

These chemical analysis techniques are well established, sensitive and accurate but they provide information only on the net concentrations of ions released. Additional techniques are necessary to visualise the crystal surfaces and deposition or nucleation of new material, and only in vitro processes can be investigated [Arends and ten Bosch, 1992]. However, chemical techniques do have the advantage that erosion of natural enamel surfaces can be investigated, since no polishing is required. In addition, this method of investigation is one of the few from which genuinely real-time data can be obtained.

2.3 Qualitative Assessment

2.3.1 SEM/TEM

Scanning electron microscopy (SEM) is a well established microscopy technique in most areas of modern science. In the investigation of dental erosion, it has been mainly used as an imaging tool, and many researchers have produced excellent, high-resolution images of eroded enamel surfaces [Meurman and Frank, 1991; Meurman et al., 1990; Rytomaa et al., 1988]. Hence, SEM is usually used as an adjunct investigation tool to other quantitative methods. However, it may also be used to help identify other phases of calcium phosphate in the hydroxyapatite crystals such as
brushite (CaHPO$_4$·2H$_2$O) and monetite (CaHPO$_4$) which form under some condition of enamel dissolution [Shellis et al., 1997].

The environmental scanning electron microscope (ESEM) is a modification of the SEM [Danilatos, 1993; Uwins, 1994]. The main differences between ESEM and conventional SEM are that the sample can be examined without coating with metal or carbon, in low vacuum and in wet conditions. The resolution and magnification afforded by ESEM are comparable to those of SEM [Prack, 1993]. To date, there are very few published reports of the application of ESEM to enamel erosion research. One shows typical images of etched premolar enamel [Cowan et al., 1996] and another shows a sequence of images of the progression of erosion in premolar enamel after 30, 60, and 90 seconds [Kodaka et al., 1993].

Energy dispersive X-ray spectroscopy, known as EDX, is a form of microanalysis that may be incorporated into SEM or ESEM. The interaction of the electron beam with the sample surface causes X-rays to be emitted by the atoms and ions in the top few micrometres of the sample surface. An electron is ejected from an inner shell of an atom, and when an outer shell electron takes the place of the missing electron, energy is emitted in the form of X-ray radiation. The X-rays are analysed and provide information on the elemental distribution in the surface. An EDX spectrum of a sound enamel surface demonstrates peaks corresponding to calcium, phosphorus, oxygen, carbon and chlorine, in addition to a small peak for silicon, which is thought to be contamination due to lubrication with silicon grease [Barbour and Rees, 2004]. The application of EDX in enamel and especially dentine erosion studies has increased recently. de-Melo et al [de-Melo et al., 2011] recently used SEM combined with EDX to evaluate the effects of a low-intensity diode laser on the dentinal chemical composition and prevention of demineralisation. Ganss et al [Ganss et al., 2010] studied the retention of tin from stannous fluoride solution on sound and eroded dentine with EDX.
and found that erosion inhibition depended mainly on the incorporation of tin in the mineralized dentine when the organic portion was preserved.

2.4 Validation of New Assessment Tools

To validate new assessment tools on the validity and sensitivity of their measurements on various stages of erosion, TMR has been the assessment tool or gold standard of choice [Elton et al., 2009; Fried et al., 2010; Pretty et al., 2004].

However for studies that require longitudinal monitoring of early enamel erosion lesions, TMR is not suitable mainly due to its destructive nature. The gold standard assessment method for erosion studies that assess early demineralisation over a period of time would ideally need to meet the following criteria:

1. A quantitative method that quantify mineral loss directly or indirectly.
2. A non-destructive method.
3. A method that is sensitive to early demineralisation.
4. A method that could potentially be used to assess unpolished, natural enamel surface.

Of the assessment methods discussed earlier in this chapter, surface hardness and ion analysis seem to be the two methods that meets the criteria although each has its own benefits and disadvantages and the choice of between the two would be a matter of reasoning and weighing of the nature and needs of a particular study.
2.5 References


CHAPTER 3

CLINICAL STUDIES OF DENTAL EROSION AND EROSIVE WEAR

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3.1 Abstract

We define erosion as a partial demineralization of enamel or dentine by intrinsic or extrinsic acids and erosive tooth wear as the accelerated loss of dental hard tissue through the combined effect of erosion and mechanical wear (abrasion and attrition) on the tooth surface. Most experts believe that during the last decade there has been a significant increase in the prevalence and severity of erosive tooth wear, particularly in adolescents. Even when erosive wear occurs in its milder forms this is a matter for concern, as it may compromise the integrity of an otherwise healthy dentition in later life. The erosive wear process is complicated and modified by many chemical, behavioural and associated processes in the mouth. If interventions are to be developed it is therefore important that in vivo methods are developed to assess the outcomes of the erosion and erosive wear processes and the effects of interventions upon them. This paper discusses potential methods of investigating erosion and erosive wear in vivo and the difficulties associated with clinical studies.

3.2 Introduction

Tooth wear has been described as presenting itself in different types: abrasion, attrition and erosion being the most common. Dental erosion has been defined as a chemical process that involves the dissolution of enamel and dentine by acid not derived from bacteria, when the surrounding aqueous phase is under saturated with tooth mineral [Larsen, 1990]. Erosion results in the softening of enamel and dentine, which, in combination with mechanical factors such as abrasion and attrition, results in accelerated, pathological wear of the teeth. Although the terms dental erosion and dental erosive wear are often considered synonymous, we will refer to this erosion-facilitated wear as “erosive wear”, reserving the term erosion only for the chemical process as defined above. The clinical condition of erosive tooth wear is recognised to have a multi-factorial aetiology [Young et al., 2008]. Together with the limitations in our
clinical detection of signs of “pure” erosive wear, this suggests that wear types are not mutually exclusive, but rather reflect overlapping zones in an etiological spectrum.

The histopathological aspects of both enamel and dentin erosion are described by Lussi et al. [Lussi et al., 2011]. The generalised pathological wear resulting from erosion may compromise the health of individual teeth and of the entire dentition.

The rate and severity of erosive wear results from the interplay between several chemical, biological and behavioural factors. Although some of these factors may be modelled in vivo or in vivo to some degree, all factors interact, and contribute to the clinical manifestation of erosive wear, only in vivo. Therefore, for a final validation of the results of in vivo and in vivo modelling, clinical studies using the natural dentition of appropriate study populations are necessary.

3.3 Prevalence of tooth wear

Tooth wear is a physiological process. Compensatory mechanisms (deposition of secondary dentine, alveolar growth and muscle adaptation) may ensure a continued functionality even with severe wear [Berry and Poole, 1976]. However, in a population with an increased life expectancy and with increased emphasis on the aesthetic and phonetic functions of teeth, severe wear may be considered pathological. Physiological tooth wear coupled with a cumulative damage profile, will result in an age-related prevalence of the condition, which has been confirmed for adults and for deciduous teeth in children in systematic reviews [Kreulen et al., 2010; van ’t Spijker et al., 2009]. A prevalence of severe tooth wear (established wear into dentin) of 3% at the age of 20 years was predicted, increasing to 17% at the age of 70 years. What proportion of the prevalence should be considered pathological is not clear, nor is the proportion of erosive wear.
3.4 Prevalence of erosive wear

Reports of the prevalence of erosive wear only vary widely, the ranges of prevalence being 6-50% in pre-school children, 11-100% in adolescents (9 to 17 years old) and 4-82% in adults [Jaeggi and Lussi, 2006]. The wide ranges may reflect actual population differences, but are more likely a reflection of different diagnostic criteria and thresholds. Considering the high reported prevalence of erosive wear, and the relatively low prevalence of severe tooth wear in general (3% to 17% from 20 to 70 years), there is reason to suspect that the rate of progression of erosive wear varies greatly, from very mild to very aggressive. At the moment the factors that determine the severity of the condition are not known, nor is it known whether one preventive approach would be suitable for all grades.

There is a consensus in the general dental and research communities that dental erosive wear has become more prevalent, but there is a lack of historical data to support this. Dutch studies, using successive cross-sectional surveys among school children in The Hague, appeared to show a dramatic increase over just 4 years (from 3% to 23%) [Truin et al., 2005; van Rijkom et al., 2002]. However, in retrospect this might be attributed to changes in diagnostic methods. An increased awareness of the problem, perhaps linked to lower caries prevalence, has been blamed for the increased reporting of erosive wear. The best indication that prevalence has truly increased is from a German study using orthodontic models: thus allowing the diagnostic methods of today to be used on historical records [Ganss et al., 2001]. This study showed an increase in 11-year-old children showing any sign of erosion from 6.3% (cohort before 1990) to 15.1% (cohort after 1990).

3.5 Incidence and progression of erosive wear

Only a few longitudinal studies have evaluated incidence (new individuals diagnosed) or progression (increase of severity) of erosive wear, predominantly in children. Dugmore and Rock [Dugmore and Rock, 2003] reported an incidence of erosive wear
of 12.3% over a two-year period in a sample of 12-year-old children. Ganss et al. [Ganss et al., 2001], using orthodontic models, observed an incidence of 18% between the ages of about 11 and 16 years. El Aidi et al. [El Aidi et al., 2010] looked at both incidence and progression of erosive wear over 3 years in 622 children of a starting age between 10 and 12 years. The incidence (new cases showing any erosive wear after 1.5 years) dropped markedly from 26.5% at 11 years to 6.4% at 14 years, indicating a degree of separation into cases (42%) and non-cases (66%) at age 15 years. Erosive wear progressed in about 30% of cases at every age level. [Lussi and Schaffner, 2000] reported that in a sample of 55 adults in two age groups (26-30 and 46-50 years), there was progression of erosive wear on facial and occlusal surfaces and of wedge-shaped defects over 6 years. The increase in the defects was more marked in the older age group.

3.6 Clinical studies

The complexity of the chemical, biological and behavioural modulating factors for dental erosion, makes the predictive validity of in vivo or even in vivo studies unclear when assessing interventions. Studies under real life conditions, on real people using their natural teeth, would be ideal but such studies are challenging and few have been conducted to date.

3.6.1 Population groups

Potential population groups in which clinical studies could be conducted can be broadly divided into 3 groups: healthy volunteers; those with evidence of current or past erosive wear; and those with aggressive erosive wear. The suitability of these groups for participation in clinical studies is discussed below.

3.6.2 Healthy volunteers

Healthy volunteers are perhaps the easiest subjects to recruit for participating in clinical studies, but there are two major problems with this type of subject. Firstly, because
these individuals have no evidence of disease it would be ethically unsound to induce significant levels of possibly irreversible erosive wear. Hence study designs should be limited to inducing an early level of demineralisation or softening that is clinically insignificant and which could be reversed or remineralised either with an oral healthcare product or by the natural exposure to saliva. Secondly, the biological and behavioural characteristics of the individual and the properties of their enamel may mean that such subjects do not respond in the same way as those with evidence of ongoing erosion. For example mature natural dental enamel may be less susceptible to erosion than enamel that has been eroded because of changes in mineral content and surface characteristics. With these caveats in mind healthy volunteers may be suitable for erosion studies that attempt to investigate changes in mineral loss or changes in surface characteristics.

3.6.3 Populations with evidence of erosive wear

Identification of individuals with existing and ongoing erosive wear could be suitable for both qualitative and quantitative studies, particularly if the aim was to arrest or slow down the existing erosive process. Evidence of erosive wear does not imply that it is still going on or gives an indication of its rate. Hence conducting studies in such groups may be somewhat unpredictable. However, this population group is the largest against which products to modulate the effects of erosion might be targeted, and should be a key focus of clinical research.

3.6.4 Populations with aggressive erosive wear

Patients with aggressive and rapidly progressing erosive wear, such as some with gastro-oesophageal reflux disease (GORD) or bulimia, might be suitable for the study of erosive wear and particularly quantitative surface loss. However, the standard care to treat the underlying condition usually involves multiple approaches, including behavioural modification and medical intervention with various drugs as well as with oral care products and this complicates the assessment of added benefit from new
products or approaches. Placebo control may not be ethically justified, although in a recent study on GORD patients a short time placebo intervention (3 weeks) received ethical approval [Wilder-Smith et al., 2009]. Generally, these population groups have the greatest need for interventions to arrest erosive wear but it is clear that preventive interventions for these extreme conditions may not be suitable for use in the widespread population.

It can be seen from the above that identification of suitable populations in which to conduct clinical studies is difficult and the conclusions that can be drawn from them are not always clear. For example, interventions that work by reducing enamel softening under mild conditions may be completely ineffective for dentine wear, in more severe conditions or in a highly abrasive environment.

3.7 Evaluation of erosion and erosive wear

Broadly, two approaches to conducting clinical studies might be taken, depending on whether the aim is to study erosion (chemical process) or erosive wear (the multifactorial clinical condition). Ideally, it would be desirable to test interventions on erosive wear but this requires either complicated modelling that takes into account multiple factors, or actual clinical trials with associated high costs in time and money. As erosion precedes erosive wear and is assumed to be the overriding factor in erosive wear, studying erosion may be expected to contribute significantly to the knowledge and prevention of erosive wear.

3.7.1 Methods of assessing erosion

For assessing erosion in isolation from erosive wear, two approaches might be employed: measurement of the amount of calcium released by an erosive challenge, or quantification of changes in the optical characteristics, roughness or hardness of the partially demineralised surface.
3.7.1.1 Dissolved or mobilized mineral

Interventions that modulate the effects of acid dissolution by modifying the tooth surface, e.g. through incorporation of fluoride or other ions, buffering acid or protecting the tooth surface, might be assessed by chemical analysis of enamel dissolution.

Two clinical trials have used a method developed in Oslo, in which labial surfaces of anterior teeth in healthy subjects are exposed to short, mild erosive challenges: 5 mL of 0.01 mol/L citric acid dripped onto the tooth surface for about 50 s [Young et al., 2006]. Calcium analysis of the run-off solution provides a direct measure of the amount of enamel dissolution. In a first study, the short-term effect of different fluoride solutions on enamel erosion was evaluated, by comparing the calcium loss due to the erosive challenge before and 5 min after the fluoride treatment [Hjortsjo et al., 2009a]. In a second study the extended effects of two fluoride treatments (SnF$_2$ and HF) were compared after 1, 7, 14 and 28 days [Hjortsjo et al., 2009b].

The model showed differences between different fluoride treatments, and a protective effect of HF treatment up to 14 days. However, there may be some concerns with the model. In the short-term study of Hjortsjö et al. [Hjortsjo et al., 2009a], a highly significant erosion-increasing effect of NaF treatment was found, and similarly in the longer term study [Hjortsjo et al., 2009b] an erosion-increasing effect of SnF$_2$. These results could not be explained, and no control data for untreated surfaces were available. The contribution of pellicle calcium to the measurement could not be determined.

Although this is a true clinical model, several key factors in clinical erosion and erosive wear are not taken into account. Most importantly, the erosive challenge is unnatural, as the natural mouth physiology of saliva and soft tissue is still excluded. The advantage over an in vivo model, where repeated erosive challenges are feasible, and erosive wear can also be assessed, is not immediately clear.
Longbottom et al [Longbottom et al., 2008] assessed the susceptibility of enamel to erosion using a luminescent calcium-ion-binding agent as a marker for surface changes due to the release of calcium after erosive challenge. Unexposed enamel produced less luminescence than enamel exposed to erosion. Significant differences in luminescence were observed between different types of erosive challenges. The method may be useful for assessing potential erosion risk in the future.

3.7.1.2 Surface characteristics

Developing instruments that can quantify subtle surface changes, such as early demineralisation and erosive softening in vivo is extremely challenging. Such instruments need to be capable of taking measurements of the natural enamel surface, with its intrinsic curvature, and of the natural dentine surface, with its persistent demineralised organic matrix. In addition, when small amounts of mineral loss are to be monitored, the tooth surface should be placed in as reproducible a position as possible for measurement, because surfaces are often inhomogeneous. Although Fosse et al. [Fosse et al., 1986] have developed an instrument for measuring microhardness in the mouth, it has not been adequately tested [Schlueter et al., 2011]. Accurate intra-oral measurement of this and other properties of tooth surfaces, such as roughness, require extensive development work.

3.7.1.3 Surface layer demineralization

In general, potential methods of qualitatively assessing early erosion are based on the optical properties of enamel or dentine. The principle of these methods is that after erosion the tooth surface is roughened and porous, so will scatter more light and transmit less than sound tissue. Methods such as laser light backscattering, previously investigated for detection and measurement of early caries lesions, might also be applicable to dental erosion [Angmar-Mansson and ten Bosch, 1987]. However, currently the two most promising methods of assessing the surface characteristics of
enamel in vivo are quantitative light-induced fluorescence (QLF) and optical coherence tomography (OCT).

### 3.7.1.4 Quantitative Light-Induced Fluorescence

QLF is a non-invasive optical technique initially developed for in-vivo measurement of early caries [de Josselin de Jong et al., 1995] and since used extensively for investigation of caries and to a lesser extent fluorosis [Pretty et al., 2006].

In order for the method to be used to measure erosion, an area with little or no surface scattering needs to be employed as a reference area to calculate relative loss of fluorescence (△F) in the test area. In practice, this might be achieved by either protecting part of the surface prior to erosion or alternatively it might be possible to use a fluid, e.g. water, which will reduce scattering in the reference area.

*In vivo* studies attempting to validate the use of QLF for quantifying acid erosion of enamel have used △Q as the outcome variable (the product of △F and lesion area). Pretty et al. [Pretty et al., 2004] demonstrated the ability of QLF to detect and longitudinally monitor erosion on unpolished enamel surfaces subjected to 30 min intervals of erosion for up to 15 h. Elton et al. [Elton et al., 2009] found a weak correlation of 0.22 between △Q and integrated mineral loss, measured by TMR, and concluded that QLF is reliable for shallow erosive lesions but becomes less consistent as erosion advances. QLF may have the potential to measure early erosion *in vivo*, particularly in its earlier stages prior to surface loss, but a significant amount of work is required to validate it clinically.

### 3.7.1.5 Optical Coherence Tomography

OCT has developed rapidly in recent years in terms of resolution, data acquisition speed, tissue penetration, contrast enhancement and delivery systems for clinical application. The method provides cross-sectional imaging by measuring the magnitude and echo time delay of backscattered light. The increased porosity of demineralised...
enamel, compared to sound enamel, results in a change in optical properties hence a difference of intensity of the reflected light can also be quantified and analysed [Amaechi et al., 2003; Popescu et al., 2008].

For dental erosion the method is based upon quantitative measurements of the backscattered light intensity at the surface, which indicates surface porosity and also depth of penetration of the region of interest, which is reduced when surface scattering occurs. Using this approach the increased porosity of demineralised enamel compared to sound enamel can be estimated (Figure 3.1). Recently OCT was used to quantify demineralisation in an in vivo study of the effectiveness of a treatment for GORD [Wilder-Smith et al., 2009]. A significantly reduced intensity of backscattered light was observed in the treatment group compared to the placebo group.

OCT provides real time imaging and does not require specimen processing. It is non-invasive and can potentially measure both surface characteristics and quantitative loss of tooth structure (Figure 3.2). Ideally, as with other techniques of this type, accurate repositioning of the probe is required so that the same area is measured at different time points. An area of no change may also be required as a reference.

3.7.2 Methods of assessing erosive wear

Objective in vivo assessment of erosive wear is difficult because surface loss generally progresses slowly and current methods have generally low resolution and require extended observation periods to detect changes reproducibly. Not least of the challenges is the identification of a stable reference from which loss of tooth substance can be gauged. In many populations erosive wear is often seen as cuspal cupping on first molars and ideally any instrumental technique that is developed should allow monitoring of these teeth.
3.7.2.1 Clinical scoring methods

An ideal scoring method for measurement of erosive wear over time should be simple to understand and use, clear in its criteria and reproducible. Many different erosion and tooth surface loss indices have been developed and used for epidemiological and clinical purposes. Most combine quantitative and qualitative criteria and rely in part on assessing the presence or absence of dentine exposure. Quantitative criteria used are objective physical measurements such as the area of worn surfaces and depth of cuspal cupping.

Eccles [Eccles, 1978] originally classified lesions broadly as early, small and advanced, with no strict criteria definitions, thus allowing wide interpretation. Later, the scoring method was refined and expanded, with greater emphasis on the descriptive criteria [Eccles, 1979]. It not only graded severity of erosion but also site of erosive wear and is considered one of the cardinal indices from which others have evolved. Smith and Knight [Smith and Knight, 1984] took Eccles’ idea a stage further and produced the Tooth Wear Index (TWI), in which all four visible surfaces of every tooth are scored for wear, irrespective of its aetiology. Millward et al. [Millward et al., 1994] made adjustments to the TWI to study erosive wear in the primary and young secondary dentitions, excluding cervical surfaces. Linkosalo and Markkanen [Linkosalo and Markkanen, 1985] utilised a qualitative score with listed diagnostic criteria and a four-point scale to grade severity of erosion. Their scoring system was then modified by Lussi [Lussi, 1996] to create an erosive wear score that has been used widely by European researchers to score labial, lingual and occlusal surfaces of all teeth except the third molars (Table 1.1).

The methods described above have been used mainly in epidemiological settings. For clinical use, Oilo et al. [Oilo et al., 1987] pioneered a different type of scoring system, with its criteria based upon treatment need but this approach requires experience for reliable use because individuals with differing clinical backgrounds could produce
differing results. Also, treatment need is not only determined by the degree of wear, but also by the patient’s subjective problem. There have also been attempts to visualise, measure and monitor the amount of worn enamel or exposed dentine indirectly on serial study casts [Fareed et al., 1990; Schlueter et al., 2005].

The most recent scoring system the Basic Erosive Wear examination (BEWE) was developed for use in general practice and to allow comparison with other indices [Bartlett et al., 2008]. Each sextant is scored independently and a score applied which is linked into the clinical management of the condition. It is hoped that this approach will be adopted internationally to allow comparisons of studies.

It is doubtful whether any of the approaches described are sensitive enough to permit wear to be monitored within a reasonable time, and they cannot be used readily to measure the rate of erosive wear. To date, there is no one method that meets all the requirements of clinical staging and longitudinal monitoring of erosive tooth wear.

3.7.2.2 Indirect profilometry

To date few studies monitoring erosive wear using indirect profilometry have been described. Two studies involved cementing a metal marker onto the palatal surfaces of upper incisors as a reference point. Erosion or tooth wear was then estimated by scanning either impressions or study casts with surface profilometry at regular intervals [Bartlett et al., 1996; Schlueter et al., 2005]. The principle of this method is to measure step height between the unchangeable reference area and an experimental area that is exposed to erosive challenges - an increasing step height in sequential measurements indicates a progression of tissue loss [Ganss et al., 2005]. One disadvantage of this approach is that it is a relatively invasive procedure.

The method has been used in a clinical trial of the effect of a bonding agent application on the wear of exposed dentin on anterior palatal surfaces [Sundaram et al., 2007b]. The majority of the subjects had suspected erosive wear. The study had a split-mouth
design and lasted for 20 months. Apart from subject attrition, the main problem was loss of the metal reference discs: 25% by 3 months, 50% by 9 months and virtually all by 20 months. The study was further complicated by the preventive measure changing the baseline surface height, thus making true dentine wear more difficult to determine. However, the difference in "wear" between resin-covered and control teeth at three months was statistically significant.

The method seems appropriate for longer-term evaluation of erosive wear. It could also be applied to enamel wear measurements. Obviously, enough inter-occlusal space is required for the metal references not to interfere with occlusion and articulation, and this application of profilometry to assessing occlusal wear may be difficult for that reason. Better retention of the references is necessary, but other materials, such as composite resin, may be considered where mechanical wear is expected to be negligible.

Other systems generate superimposed three-dimensional (3D) digital images by profiling consecutive dental casts with a null contact profiler [Pesun et al., 2000; Pintado et al., 1997] or with an optical 3D sensor [Mehl et al., 1997]. Direct 3D scanning of a tooth or surface using the systems developed for CAD CAM preparation of restorative materials or modifications thereof also represent a potential solution to the measuring tooth wear in vivo. Again, a significant amount of work may be required before these systems can be adapted for this task.

### 3.7.2.3 Measurements of enamel layer thickness

Currently, two clinically applicable methods for directly measuring enamel layer thickness are available - OCT (Figure 3.2), ultrasound [Huysmans and Thijssen, 2000] and more recently high frequency ultrasound [Hughes et al., 2009]. For methods measuring the thickness of the enamel layer, the problem of finding a stable reference is solved, as the amelodentinal junction (ADJ) is stable. However, the thickness of the enamel layer is very location-dependent and in particular it becomes thinner towards
the cervical region, so the main problem is that of ensuring that the same measurement site is used at each time point. Using an elaborate method of holes drilled into an impression of the subject's jaw, to ensure good OCT-probe positioning, thickness changes within the µm range have been measured [Wilder-Smith et al., 2009]. Enamel layer thickness reduction was the main outcome parameter in this study on 30 GORD patients, which lasted only 3 weeks. The measurement error was reported to be well below 10%, and the threshold for wear detection was in the range of 5-10 µm. After 3 weeks a significant wear reduction of about 50% (7.2 µm vs. 15.3 µm) was seen. Without such repositioning, ultrasound was estimated to have a measurement resolution of more than 300 µm in a simulated clinical set-up [Louwerse et al., 2004].

Recently, Hughes et al [Hughes et al., 2009] managed to achieve an axial resolution of 180 µm and a spatial resolution of 110 µm with a custom-designed, high frequency (35 MHz) piezo-composite ultrasound transducer. However, there has not been any clinical application of this technology to date.

A few questions remain regarding the above mentioned OCT measurement technique. For instance, the average enamel layer thickness reported by Wilder-Smith et al. [Wilder-Smith et al., 2009] was more than 2 mm - this seems extremely high for severe wear patients. Also, although the authors reported that measurement was “relatively unproblematic”, it appears to be time-consuming and complex, and this might limit its use in larger groups. Because the method relies on the ADJ as a reference, it cannot be used to measure tissue loss once the dentin has been reached.

The combination of aggressive erosive wear with a very precise measurement allowed for a very short trial duration in this last study. As this example involved a pharmaceutical treatment for a medical condition it is likely that patients would continue the therapy and a longer evaluation may not be needed. In preventive treatments of erosive wear alone, where patients may have no symptoms, compliance may be more of an issue, and longer studies would probably be needed to assess the realistic
therapeutic effectiveness. However, the measurement method used is suitable also for such longer studies.

3.8 Feasibility aspects of clinical trials

In clinical trials evaluating (prevention of) natural erosive wear, two variables are extremely important in determining the size, duration and therefore the feasibility of the study: the rate of lesion progression and the variation in this rate. For "mild" erosive wear, such as usually reported in surveys in children and adolescent, the rate in μm enamel per year is never reported, and there are only visual scoring systems as measures. El Aidi et al. [El Aidi et al., 2010] found that about 30% of erosive wear cases showed progression over 1.5 years, suggesting that variation in rate is probably high. We recently performed a sample size calculation for an erosion prevention study in such a population of 12-year-olds with early erosion, based on the clinical scoring method only. For a treatment with an expected effect of 25% wear reduction, the estimated group size was about 800, for an 18 months study. To arrive at a sample of this size 6000 12-year-olds would need to be screened.

There are a few reports on rates of progression in aggressive erosive wear cases. Bartlett et al. [Bartlett et al., 1997] reported a palatal enamel wear rate of about 75 μm per year, with a range of 35-215 μm, while healthy control subjects lost about 7 μm per year. The dentine wear rate of control teeth in the dentine bonding study was about 100 μm per year [Sundaram et al., 2007a]. The enamel wear rate in control subjects in the GORD clinical trial was about 250 μm per year [Wilder-Smith et al., 2009]. Here the reported variation was much lower. We estimate that the 95% confidence interval for the rate was 175 to 350 μm.

In caries research it has taken a long time to move away from the cavitated lesion as the only relevant outcome of caries studies and to include earlier stages of the disease - non-cavitated lesions. However, in caries progression single episodes of demineralisation are not considered relevant outcomes, as caries lesions only develop
when the balance between episodes of demineralisation and episodes of remineralisation is shifted towards demineralisation for a significant period of time. In research into erosion or erosive wear the matter tends to be approached from the other side - we have studied mainly dental erosion (short episodes of acid attack), but less often the clinical outcome of erosive wear. Although there is evidence that remineralisation after an erosive attack is minimal, it is unclear to what level such surfaces will remineralise, if at all, and how mechanical wear modulates this process. It is not proven that preventive measures that reduce dental erosion will equally reduce erosive wear. Ideally the validity of an isolated and early measurement of susceptibility needs to be shown in a longitudinal study evaluating the clinically relevant outcome of tissue loss.

3.9 Conclusion

Clinical trials involving patients with mild erosive wear may already be feasible using existing clinical scoring systems, but they would require large groups and several years of follow up. An alternative would be to assess dental erosion (surface changes) in such a study, but validity would then be an issue. Clinical trials involving patients with aggressive erosion have been performed, but the results may not be suitable for extrapolation to other populations.

It is clearly important that appropriate clinical methods are developed and validated to assess erosion and erosive tooth wear in vivo. New diagnostic technologies may provide solutions to this challenging task but a significant amount of development is still required.

As discussed above, OCT and QLF are able to provide real time data and have been used in clinical investigations to study caries and advanced erosion lesions. However, little has been done to evaluate their potential in the assessment of early erosion. The subsequent three chapters describe the in vivo validation work of these systems to determine their sensitivity and limitations in the assessment of early enamel erosion.
3.10 References


3.11 Figures

Figure 3.1

Figure shows the OCT B-scans of an upper incisor taken at three different intervals; baseline, after 10 and 60 min of erosive challenge in orange juice. As the erosive interval progressed, surface change in the form of increased surface light backscattering was visualised on the surface of the non-coated side of the enamel.
Figure 3.2

Figure shows the OCT B-Scan of an incisor after being subjected to 24 hours of erosion challenge in orange juice. The right half of the sample was coated with varnish while the other half was left exposed to orange juice. A step change of 130 µm in depth and a superficial layer of increased light backscattering were observed on the exposed side.
CHAPTER 4

MATERIALS AND METHODS
4.1 In Vitro Set up

4.1.1 Sample preparation
A convenience sample of 12 extracted upper or lower human central incisors, caries free with no visually obvious fluorosis, were used in this study. The teeth were collected by the Kerala Dental School and were stored in thymolised water before the start of the study. Each tooth was fully embedded in a separate cold cured methyl-methacrylate resin block with dimensions of 10 mm x 10 mm x 30 mm, except for a 5 mm x 5 mm window at the middle third of its labial surface. The incisors were mounted such that the exposed labial surface was at least level with or not more than 1mm higher than the adjacent resin. This was to ensure optimum contact with the orange juice and to keep the height variation consistent with the depth of field of QLF.

4.1.1.1 Coating of the Reference Area
The material chosen to coat the reference area for this study was Xeno® V (DENTSPLY UK), a mild one step, self-etch adhesive. A pilot study was carried out to determine whether the presence of this adhesive altered the OCT backscattered intensity and the fluorescence of QLF. 5 lower incisors were each fully embedded in separate cold cured methyl-methacrylate resin blocks with dimensions of 10 mm x 10 mm x 30 mm, except for a 5 mm x 5 mm window at the middle third of its labial surface. An OCT mesio-distal B-scan across the middle of the window was performed and a QLF image was taken for each tooth after being exposed to 20 seconds of compressed air that was fixed 10cm away from the resin block.

The window was then coated with a layer Xeno® V according to the manufacturer’s instructions which did not include an additional step of etching with phosphoric acid. Another OCT scan and QLF image was taken at the same position a day later. The following data for each measurement were generated:
i) OCT backscattered intensity of depth 20 μm up to 200 μm from the surface

ii) The mean fluorescence of the window

Paired T Test was used to compare the results of both days and no significant differences were detected between the OCT (P = 0.33) and QLF (P = 0.45) measurements.

Therefore, in the actual study, half of the exposed labial surface, i.e. approximately 2.5 mm x 5 mm, was protected with a non-residue masking tape while the other half was being coated with the adhesive. The application of the bonding agent was performed according to the manufacturer’s instructions which did not include an additional step of etching with phosphoric acid. This adhesive coated area served as the reference area.

After the application of the Xeno® V (DENTSPLY UK) was completed, the protecting masking tape was removed from the other half for exposure to the erosive challenge. After the application of the adhesive, baseline surface microhardness (SMH) measurements of the exposed area were recorded and baseline QLF and OCT images were taken of both the exposed and non-exposed areas.

4.1.2 Erosion Cycle

The teeth mounted in the resin blocks were then suspended using plastic rods in a commercially available orange juice (ASDA Orange Juice from Concentrate) (pH 3.8 ± 0.1). Two groups of six teeth were suspended, each in 500 ml of orange juice and the orange juice was gently stirred with a magnetic stirrer at a fixed revolution rate per minute (rpm) at room temperature (Figure 4.1). The pH of the orange juice was monitored with a pH meter (Orion 4-Star Plus pH / DO Portable Meter) throughout the erosion cycle and the pH recorded every 5 minutes. After every 10 minutes, for up to a total of 60 minutes, the teeth were removed from the orange juice and rinsed under a reservoir of running water for 1 minute to remove excess acid from the surface of the teeth and then immersed in a beaker of 100ml of distilled water. With rinsing, the erosion process would be expected to be retarded, if not stopped, so as to avoid non-uniformity of acid exposure time during measurement. Each sample was removed
separately from immersion and then dried for 20 seconds with compressed air, which was fixed at 10cm away from the resin block. Each specimen was first scanned with OCT, followed by imaging with QLF and lastly surface microhardness indentation. Each sample was then returned into distilled water. The process of scanning with OCT plus imaging with QLF took 40 ± 5 seconds. After measurement of all samples had been taken, they were then re-immersed in a fresh batch of 500ml stirred orange for 10 minutes and the process of measurement repeated. The process continued until the samples were exposed to a cumulative period of 60 minutes of erosion. The orange juice was changed after every 10 minute period of erosive challenge.

4.1.3 Surface microhardness

The micro-indenter, Microhardness Tester FM-700 (Future-Tech Corporation, Japan) was used in the study. The resin blocks were placed flat on the translation stage and fixed at a reproducible position with the precision vice of the micro-indenter. A surface area of approximately 1 mm x 1 mm perpendicular to the direction of the load of the indenter was identified in the uncoated half of the enamel window for indentation. The identification of the perpendicular measurement area was made by ensuring that the aiming beam of the micro-indenter was in focus before the loading was performed. Micro-indents were made using a Knoop diamond indenter, with a load of 25 g applied for 5 seconds.

Five indentations approximately 100µm apart were made during each measurement time point at the identified measurement area. The horizontal lengths were measured and the Knoop numbers ($KHN$) were calculated and averaged. Although care was taken to ensure that indentations were made on a surface that was perpendicular to the direction of loading, due to the inherent curvature of a natural and unpolished enamel surface and the presence of local irregularities such as perikymata, some asymmetrical indents were observed and were not included in the analysis. In these cases, additional indents were made. The outcome measure was expressed as the percentage of
surface microhardness change ($\Delta SMC$), based on the differences between Knoop hardness numbers at baseline, $KHN(t_0)$ and the subsequent erosion intervals, $KHN(t)$. $\Delta SMC$ was calculated as:

$$\Delta SMC(t) = 100 \left( \frac{KHN(t) - KHN(t_0)}{KHN(t_0)} \right)$$

### 4.1.4 QLF

An in-house QLF set up was developed and built. Illumination was based on a custom-made 395 nm-LED array (B5-437-CVD, Roithner Lasertechnik GmbH, Austria), designed in a ring configuration around the imaging lens to ensure uniform illumination over the sample. A blue band pass filter centred at 425 nm, was fitted in front of the array to prevent any light above 450 nm emitted from the LED reaching the sample. Images were captured with a triple charged coupled device (3CCD)-colour camera of 1014 x 768 pixel resolution (HV-F31, Hitachi Kukasai Electric U.K. Ltd.) A 50 mm focal length-imaging lens (Fujinon HF50HA-1B, Fujifilm U.K. Ltd.) with a 10 mm-extension was attached to the camera and a 515 nm long-pass yellow filter (OG-515, Edmund Optics Ltd, U.K.) was used to filter the fluorescence light emitted by the sample.

Prior to the study, the optimum intensity of the incident light and settings of the 3CCD camera was configured so as to acquire a setting configuration that produced a high level of fluorescence at the beginning of the study. Histograms of the range of the intensity of the green fluorescence detected in each of the samples were generated to ensure that the green fluorescence at the beginning of the study was in the middle of the range detectable by the 3CCD camera (Figure 4.2).

The images were taken in a dark enclosure and captured with bespoke software which enabled repositioning of the samples, using the video camera, at the various measuring time points with respect to baseline. An outline of the baseline image in the form of an overlay was superimposed on the live video of the image to facilitate alignment before an image was captured. Optimum light intensities and camera
settings were determined to ensure that the fluorescence images from the sample were within the detection dynamic range of the camera and of maximum contrast.

A program was written in MATLAB (The MathWorks Inc, USA) to analyse the images. Images from each specimen at the various erosion intervals were aligned so that representative regions of interest (ROI) of the same location and dimensions throughout the erosion interval points for the exposed and non-exposed areas could be drawn.

The mean pixel value of the green channel was obtained for the defined ROI for the exposed, \( F_E(t) \), and non-exposed areas, \( F_{NE}(t) \). All images were analysed blind to the erosive challenge intervals, \( t \). The percentage loss of fluorescence of the exposed area at each erosion interval, \( \Delta F(t) \), was calculated as follows:

\[
\Delta F(t) = 100 \left[ \frac{F_E(t)}{F_E(t_0)} - \frac{F_{NE}(t)}{F_{NE}(t_0)} \right]
\]

where \( t_0 \) is the baseline time point.

### 4.1.5 OCT

A commercially available OCT system (OCS1300SS, Thorlabs Ltd, UK) was used to capture cross sectional images of the exposed window of the tooth surface. The OCS1300SS uses Fourier Domain technology and incorporates a broadband, frequency swept laser centred at 1325 µm. It has an axial resolution of 9 µm and transverse resolution of 15 µm in air, according to the manufacturer. The hand probe was mounted with the beam facing downwards. The samples were placed on a translational stage perpendicular to the hand probe. The stage was fixed with a repositioning jig that enabled the sample to be repositioned in the same position and alignment at the different measurement time points.

The Thorlabs Swept Source OCT software was used for image capturing and for the control of the OCT settings and light beam. The light beam was configured to scan a
length of 5mm in the mesio-distal direction (x-axis) of the labial surface of the sample (Figure 5.1) and at an axial depth of 3 mm. The inciso-cervical position of the light beam on the tooth surface (y-axis) was located at a cross section with the least observed specular reflection. The (x, y) coordinate of the light beam for each sample was recorded for replication at consecutive measurement time points. The distance of the tooth surface to the probe was determined with the most convex area of the labial surface of the tooth at 1.0 ± 0.1 mm from the top of the display window of the image capture software. The maximum amplitude was calibrated with an eroded enamel sample at the beginning of each study day and the dynamic range of the OCT light was maintained to be around 20 – 30 decibels (dB). Background noise was removed before the acquisition of each image.

A program was written in MATLAB (The MathWorks Inc, USA) to analyse the changes of the OCT backscattered light intensity in time. The B-scans of each sample from the different measurement time points were aligned and a similar region of interest was selected for all seven measurement time points. This region of interest consisted of 150 A-scans in each of the exposed and non-exposed areas, as shown in Figure 5.2. The curvature of the tooth surface in the selected region of interest was compensated by aligning the peak of the backscattered intensity rise occurring at the enamel-air interface in each A-scan along the same horizontal pixel line of the B-scan. A mean A-scan was then generated from the selected 150 A-scans for both the exposed and non-exposed area (Figure 5.2).

The outcome measure for OCT was expressed as the mean percentage difference of decay $\Delta D$, between the exposed and non-exposed area. The decay, $D$, is the relationship of the attenuation of backscattered light between two optical depths of an OCT A-Scan and is represented by the function below,

$$D = \frac{I_{\text{plateau}}}{I_{\text{superficial}}}$$
$I_{\text{superficial}}$ is the intensity of backscattered light at or immediately below the tooth-air interface where demineralisation had occurred. The chosen superficial optical depths for this study were 0 µm (tooth-air interface), 10 µm, 20 µm, 30 µm, 40 µm and 50 µm below the tooth-air interface (Figure 5.3). With the refractive index of enamel being 1.62, these chosen levels translated to the physical depth of 0 µm, 6.25 µm, 12.5 µm, 18.75 µm, 25 µm and 31.25 µm below the tooth-air interface respectively. $I_{\text{plateau}}$ is the intensity of the backscattered light where the A-scans had reached a plateau and were assumed not affected by the erosive challenge. It was observed to be at the optical depth of 150 µm for this study (Figure 5.3).

$\Delta D(t)$ at each measurement time point, $t$, is represented by the function below:

$$
\Delta D(t) = 100 \left[ \frac{D_E(t)}{D_E(t_0)} - \frac{D_{NE}(t)}{D_{NE}(t_0)} \right]
$$

where $D_E$ is $D$ of the exposed area, $D_{NE}$ is $D$ of the non-exposed area, $t_0$ is the baseline time point and $t$ is the erosive challenge time point.

### 4.1.6 Statistical Analysis

The STATA\textsuperscript{TM} 10.1 (Statacorp, Texas) statistical program was used. Multiple linear regression analyses were performed, taking into account the clustering of samples using robust variance estimates, to ascertain whether time related changes were detected with the three instruments.

When a significant time-related change was found for an outcome measure, paired $t$-test was performed to identify the detection sensitivity, which is defined as the shortest subsequent time interval when a significant difference was detected.
4.2 **In Situ Set Up**

The study design of the clinical portion of the study is described in detail in Sections 7.4.1 to Sections 7.4.5. This chapter describes only the laboratory measurement methods.

4.2.1 **Enamel samples**

80 intact, caries-free human incisor teeth that were recently extracted from patients aged 18 or over, of either gender, were collected following ethical approval. Sample size calculation was performed based on Hooper et al’s study [Hooper et al., 2004] as described in section 7.4.3. All teeth were soaked in 20,000 ppm sodium hypochlorite to water solution for at least 24 hours as a disinfection procedure, and then scraped clean of any remaining tissue with a scalpel. The teeth were then washed in distilled water. The teeth were randomly divided into 4 groups ($n = 20$) as described below.

- **Group A** – Enamel surface not polished (natural surface), exposed to the oral environment for 30 days prior to acid challenge.
- **Group B** – Enamel surface not polished (natural surface), exposed to the oral environment for 15 days prior to acid challenge.
- **Group C** – Enamel surface not polished (natural surface), no prior exposure to the oral environment prior to acid challenge.
- **Group D** – Enamel surface polished, no prior exposure to the oral environment prior to acid challenge.

4.2.1.1 **Preparation of natural-surface enamel samples**

The coronal part of the tooth was sectioned from the root using a water cooled high speed diamond saw, Microslice II (Ultratec Manufacturing Inc, Santa Ana, USA). The coronal sections were then further sectioned to an area of approximately 4 mm x 4 mm. The flattest part of the specimen (labial surface) was positioned next to the cutting
blade. The blade was then retracted 3.5 mm whereupon a cut was made, giving each specimen a uniformed height of 3.5 mm to allow for sufficient dentine underneath the enamel. The lingual surface was designated as the working surface. Each section was mounted in Stycast resin (Stycast; Hitek Electronic Materials, Scunthorpe, UK), with the natural surface facing upwards so as to avoid any contact of the resin with the natural surface and the resin was left overnight to set.

4.2.1.2 Preparation of polished-surface enamel samples
Specimens that were polished flat were sectioned as above and mounted in resin with the natural surface face down in the mould. Once the resin had set, the specimens were removed from the mould and the backs were polished using silicon carbide discs (p1200) so that it would sit flat in a polishing jig. The labial surface was then polished flat using silicon carbide discs (p1200), followed by slurry of 3 µm silica powder in deionised water and finished with slurry of 0.3 µm alumina powder in deionised water. The specimens were ultrasonicated in deionised water in between each polishing stage to remove any powder debris.

4.2.1.3 Preparation of the enamel surface (bonding agent and laser indents)
The enamel surfaces were divided into 3 equal portions of 3 mm x 1 mm, with the middle third serving as the area to be exposed to acid challenge and the right and left thirds as the reference areas.

Due to the inherent assessment constraints of the surface microhardness method, an approximately 2mm x 1 mm area of the exposed area which was within 300µm of height variation was identified using a non-contact profilometer (Proscan2000, Scantron, UK). These identified areas were recorded by means of measurement from the edges of the samples and three laser indents (Figure 4.3) in triangular configuration were made to locate the area. The laser indents were made using a Er:YAG laser, Fidelis 320A (Fotona) with the following configuration: Very Short Pulse (VSP), 600milliJoule, 2 Hz for 2 seconds.
The two reference sides, including the laser indents, were coated with the dentine bonding agent, Prime and Bond NT, (Dentsply, UK), while leaving the centre area of approximately 1 mm (X-axis) x 3 mm (Y-axis) exposed (Figure 7.1). Between measurements, the samples were kept in a humidified Eppendorf holder.

4.2.2 Measurement of samples

The intra-oral appliances carrying the samples were returned to the study site at least 1 hour after the last acid challenge of each day. Prior to performing any measurements on the enamel samples, the appliance were sprayed with a 70% ethanol solution, left for 2 minutes and then rinsed under running water. Instead of using a soft bristle toothbrush to remove any debris and plaque, as was done during Phase I of the study, the surface of the enamel samples were wiped with 0.2% Chlorhexidine Gluconate using cotton pellets. This was necessary to avoid tampering with the softened eroded surfaces.

Following disinfection, the enamel samples were removed from the appliance and placed in humidified Eppendorf receptacles prior to being measured using surface microhardness, quantitative light fluorescence (QLF) and optical coherence tomography (OCT).

4.2.2.1 Surface microhardness

The microindentor, Microhardness Tester FM-700 (Future-Tech Corporation, Japan) was used in the study. A triple charged coupled device (3CCD)-colour camera of 1014 x 768 pixel resolution (HV-F31, Hitachi Kukasai Electric U.K. Ltd) was attached to the microindentor and was operated via bespoke software.

The resin blocks with embedded enamel samples were placed flat on the translation stage and fixed at a reproducible position with the precision vice of the microindentor. A surface area of approximately 1 mm x 1 mm, perpendicular to the direction of the load of the indenter, was identified at the uncoated middle third of the samples for
indentation. The identification of the perpendicular area was achieved by ensuring that the aiming beam of the micro-indenter was in focus before loading was performed. Micro indents were made using a Knoop diamond indenter, with a load of 25 g applied for 5 seconds.

At the identified area, 5 indentations approximately 100µm apart were made during each measurement time point. Each indent made was observed and imaged under 50x magnification.

Although care was taken to ensure that indentations were carried out on a surface that was perpendicular to the direction of loading, due to the inherent curvature of a natural and unpolished enamel surface and the presence of local irregularities such as perikymata, some severely asymmetrical indents were observed and were not included in the analysis. In these cases, additional indents were made.

A program was written in MATLAB (The MathWorks Inc, USA) to measure the indents. The person performing the measurement of the indents was blinded to the session of acid challenge of the indents imaged. Due to the inherent natural curvature of the surface enamel, some of the sides and points (angles) of the rhomboidal-shaped indents were not apparent / obvious, resulting in asymmetrical looking indents. On these occasions, the program was used together with a screen protractor to measure the imaged indents from the various different measurement time points. A rhomboidal-shaped screen overlay with the dimension of the indent of the Knoop indentor (longitudinal angle of 172.50° and transverse angle of 130 °) was built using a screen protractor, MB-Ruler 4.0 (MB-Softwaresolutions). This overlay was placed over asymmetrical indents in order to be able to project the length of the horizontal diagonal of the indentations.

The horizontal lengths of the 5 indents were averaged and the Knoop numbers (KHN) were calculated. The outcome measure was expressed as the percentage of surface
microhardness change ($\Delta SMC$), calculated based on the differences between Knoop hardness numbers at baseline, $KHN(t_0)$ and the subsequent erosion intervals, $KHN(t)$. $\Delta SMC$ was calculated as:

$$\Delta SMC(t) = 100 \left( \frac{KHN(t) - KHN(t_0)}{KHN(t_0)} \right)$$

4.2.2.2 QLF

The same QLF set up used as described in Chapters 5 and 6 were used in this study.

Samples removed from the Eppendorf receptacles were dried under compressed air for 20 seconds. A customised mount for the enamel samples was placed in a dark enclosure and QLF images of the samples were taken in this enclosure. The images were captured with bespoke software that enabled video repositioning of the samples (where there is an semi-automated alignment of images) from different measurement time points. Optimum light intensities and camera settings were determined to ensure that the fluorescence images from the sample were within the detection dynamic range of the camera and of maximum contrast.

The program written in MATLAB (The MathWorks Inc, USA), described in Chapters 5 and 6 for analysing data of the in vitro study, was modified to manage two reference areas instead of one. Images from each specimen at the various erosion intervals were aligned so that representative regions of interest (ROI) of the same location and dimensions throughout the erosion intervals for the exposed and non-exposed area could be drawn.

The mean pixel value of the green channel was obtained for the defined ROI for the exposed, $F_E(t)$, and non-exposed, $F_{Ne}(t)$, areas. All images were analysed blind to the erosive challenge intervals, $t$. The percentage loss of fluorescence of the exposed area at each erosion interval, $\Delta F(t)$, was calculated as follows:
\[ \Delta F(t) = 100 \left[ \frac{F_E(t)}{F_E(t_0)} - \frac{F_{NE}(t)}{F_{NE}(t_0)} \right] \]

where \( t_0 \) is the baseline time point.

### 4.2.2.3 OCT

The OCS1300SS OCT system (Thorlabs Ltd, UK) was used to capture three-dimensional OCT data from the exposed area of enamel samples. The OCS1300SS uses Fourier Domain technology and incorporates a broadband, frequency swept laser which was centred at 1325 µm. It has an axial resolution of 9 µm and transverse resolution of 15 µm in air.

The hand probe was mounted with the laser beam facing downwards and a customised mount for the enamel samples was positioned perpendicularly below the hand probe (Figure 7.4). Samples removed from the Eppendorf receptacles were dried under compressed air for 20 seconds. Enamel samples with natural surfaces were slid flat in the trough of the mount while the samples with the polished surface were placed at an angle in the trough. This was necessary for the samples with polished surface to minimise surface specular reflection. This mount provided a definite stop for the repositioning of the samples during the various measurement time points. The Thorlabs Swept Source OCT software (Thorlabs Ltd, UK) was used to control the laser to scan a 3.5 mm (X axis) x 0.5 mm (Y-axis) area of the enamel sample. The position of the laser beam was driven using an inbuilt X-Y galvanometer. The 3.5 mm (X axis) section includes the 1 mm exposed area in the centre, flanked by the non-exposed areas on either side (Figure 7.5). The optimal location of the 0.5 mm (Y-axis) in the exposed 3 mm was determined during baseline measurement. The choice of the position was based on an area with the least specular reflection. The X-Y coordinate of the laser beam for each specimen as shown in the Thorlabs Swept Source OCT software was recorded and used for repositioning of the laser beam in subsequent measurement time periods.
The distance of the hand probe from the surface of the specimen was determined with the most convex area of the labial surface of the tooth at 1.0 ± 0.1 mm from the top of the display window of the image capture software. The transverse resolution of the X-axis was set at 1024 pixels in 3.5mm and Y-axis at 24 pixels in 0.5 mm. The axial resolution of the Z-axis was set 512 pixels in 2.01mm.

The maximum amplitude was calibrated with an acrylic sample after the measurement of every eight samples. The dynamic range of the OCT light was maintained to be around 20 – 30 decibels (dB). Background noise was removed before the acquisition of each image. The power output of the laser was also monitored and recorded after every 8 measurements using a power meter (Figure 7.4).

The program written in MATLAB (The MathWorks Inc, USA) described in Chapter 5 and 6 for analysing data of the in vitro study was expanded to analyse the three dimensional data. Provisions were added to allow simultaneous cross-sectional (B-Scans) and en-face views (C-Scans) of the scanned data (Figure 7.6) and to threshold out the confounding signals from specular reflection. The C-scans of each sample from the different measuring time points were aligned and a similar region of interest of the exposed area was selected for all the six measuring time points.

Although the location of the 0.5 mm area of the Y-axis was carefully chosen to minimise the inclusion of specular reflection, it was not possible to avoid it completely. This specular reflection is particularly strong for normal incidence and the intensity varies markedly with angle of incidence. Fried et al [Fried et al., 2002] found that the intensity in this region is approximately 20dB higher than the scattering intensity in other areas and therefore masks any information about scattering at or just below the tooth surface. Hence they implemented a threshold level to their data. For this study, the selected regions of interest on the C-scans were projected onto the respective B-scans for the determination of the threshold levels (Figure 7.7). The threshold level was adjusted until all A-scans with columnar artefacts resulting from specular reflection...
were removed (Figure 7.8). The final selected region of interest consisted of 8000 ± 300 of A-scans. The curvature of the tooth surface in the selected region of interest was compensated for by aligning the peak of the backscattered intensity rise occurring at the enamel-air interface in each A-scan along the same horizontal pixel line of the B-scan. A mean A-scan was then generated from the selected 8000 ± 300 A-scans for the exposed area.

4.3 References

4.4 Figures

Figure 4.1

Six incisors were suspended each in 500 ml of orange juice and the orange juice was gently stirred with a magnetic stirrer at a fixed revolution rate per minute (rpm) at room temperature.
Figure 4.2

Histograms of the range of green channel fluorescence detected on three samples prior to acid challenge. The green channel fluorescence profile A and C are examples of profile accepted while teeth with profiles similar to profile B were not included in the study as they were already appearing dark at the beginning of the study.
Figure 4.3

Figure shows illustrations generated by the non contact profilometry. Figure A and B shows example of specimens that were included in the study. An area with the dimension of approximately 2mm x 1 mm area of the centre exposed area which is within 300µm of height variation was identified. Three laser indents were made using a Er:Yag laser were made to mark the area. Figure C and D are examples of samples that were not included in the study as they were too curved and not suitable for indentation with surface microhardness.
CHAPTER 5

MEASURING EARLY ENAMEL EROSION WITH QUANTITATIVE LIGHT-INDUCED FLUORESCENCE (QLF) AND OPTICAL COHERENCE TOMOGRAPHY (OCT): AN IN VITRO VALIDATION
5.1 Abstract

To date, the measurement of early erosion (before bulk loss) has mostly been performed with techniques such as surface microhardness and transverse micro-radiography and therefore been limited to in vitro studies. Optical Coherence Tomography (OCT) and Qualitative Light-Induced Fluorescence (QLF) are non-invasive diagnostic tools which have been used in clinical investigations to study caries [Al-Khateeb et al., 1998; Fried et al., 2011] and advanced erosion lesions [Wilder-Smith et al., 2009] but little has been done to evaluate their potential in the assessment of early erosion. The change in optical properties of early eroded enamel can potentially be quantified by these two optical techniques and used in the clinical quantification of early demineralisation in enamel. Nevertheless, before that, in vitro validation of these systems is needed to determine their sensitivity and limitations.

Objectives: To evaluate the sensitivity of the QLF and OCT in detecting early dental erosion in vitro.

Methods: A convenience sample of 12 human incisors were embedded in resin except for a 5mm x 5mm window of the buccal surface. A self-etching adhesive, Xeno® V (DENTSPLY) was applied to half of the window while the other half was left exposed. After the application of the bonding agent, baseline measurements were taken with QLF, OCT and surface microhardness. Samples were then immersed in a commercially available orange juice (pH 3.8 ± 0.1, mean ± standard error) for a total of 60 minutes and measurements taken every 10 minutes within this period. Teeth were removed from the orange juice, rinsed under running water and dried for 20 seconds with compressed air at a fixed distance before each measurement was taken. A QLF set up was built based on a 395 nm-LED ring array with a blue band pass filter for illumination and a 3-CCD colour camera with an imaging lens and a 515 nm-long-pass yellow filter for detection. The loss of light fluorescence intensity in time was compared between the exposed and non-exposed areas from each sample. A commercially
available OCT system (OCS1300SS, Thorlabs Ltd, UK) was used to capture cross sectional images of the window. The intensity of backscattered light from the various optical depths of exposed and non-exposed areas were analysed. A Knoop indenter with a load of 25 g and dwell time of 5 s was applied on the exposed area and the recorded Knoop Hardness Number analysed. Multiple linear regression and paired t-test were used to compare the change of the outcome measures at the different erosion intervals with respect to baseline.

**Results:** Measurements from all three instruments demonstrated significant dose responses with erosive challenge (P < 0.05). All three instruments demonstrated a detection threshold of 10 minutes. Thereafter, surface microhardness demonstrated significant changes after every 10 minutes of erosive challenge. QLF demonstrated significant difference in fluorescence change only at four other erosive intervals (20, 40, 50 and 60 minutes) while OCT demonstrated significant difference in decay of backscattered intensity at only two others (50 and 60 minutes).

**Conclusion:**

It is concluded that:

1. Surface microhardness, QLF and OCT were able to detect demineralisation after 10 minutes of erosive challenge *in vitro*

2. Surface microhardness, QLF and OCT could be used to monitor the progression of demineralisation in early enamel erosion *in vitro* but QLF and OCT at a longer erosive interval compared to surface microhardness.
5.2 Introduction

Dental erosion is defined as a chemical process that involves the dissolution of enamel and for dentine by acid not derived from bacteria [Larsen, 1990]. The source of acid could either be extrinsic or intrinsic, with extrinsic sources being mainly dietary and intrinsic being regurgitated gastric hydrochloric acid – often associated with gastro-oesophageal reflux disease (GORD). If dental erosion is not diagnosed and arrested in its early stages it can quickly progress and result in a severely affected dentition that will require complex restorative and orthodontic management [Amaechi et al., 2004]. Dentine hypersensitivity may also be a presenting feature of erosive wear.

Depending on the aetiology, the recommended management of this condition includes behavioural and dietary modifications [Milosevic and O'Sullivan, 2008], consumption of modified beverages [Grenby, 1996; Hughes et al., 2002] that are claimed to have less erosive potential, use of fluoride preparations with polyvalent metal cations, fluoridated varnish and in some cases medical attention [Wilder-Smith et al., 2009]. In order to investigate the efficacy of any of these preventive or management therapies, not only is the quantification of the degree of demineralisation important, the ability to longitudinally monitor the progress of the erosion lesion over the period of the therapy is also necessary.

Various clinical indices have been designed to detect and quantify tooth surface loss due to erosion from the other causes, for example Eccles and Jenkins’ [Eccles and Jenkins, 1974], Lussi’s Index [Lussi, 1996], Larsen’s index [Larsen et al., 2000] and recently the Basic Erosive Wear Examination (BEWE) index [Bartlett et al., 2008]. Most indices were designed with the clinical diagnosis, recording and monitoring of erosive wear lesions as the main focus. These indices rely on subjective clinical descriptions such as “absence of developmental ridges”, “smooth, glazed enamel”, “no distinct loss of original morphology “ [Larsen et al., 2000; Lussi, 1996] and “initial loss of surface texture” [Bartlett et al., 2008] as indicative of erosive wear. Quantification of the
affected areas were made as estimates of percentages or fractions of the whole tooth surface. As these indices use few score levels and broad quantification criteria, they have been later been adapted for epidemiological use [Arnadottir et al., 2010; Caglar et al., 2011; van Rijkom et al., 2002].

However, there have been disagreements with regards to the measurement validity of these indices as it is not always possible to rule out confounding effects from other mechanisms of tooth wear. The broad measurements of the clinical presentations by percentages of area and depth also made it impossible to monitor minor progression of detected lesions [Milosevic, 2011].

If the detection, measurement and monitoring of tooth surface loss due to erosion (erosive wear) with clinical indices is plagued with low sensitivity and validity, the detection of early erosion with clinical indices would be very difficult.

5.2.1 Measurement of early erosion lesion

To quantify and monitor early enamel erosive lesions, direct quantitative evaluations of the degree of demineralisation can be undertaken with transverse microradiography (TMR) which quantifies mineral loss [Amaechi et al., 1998]. Indirect evaluations of erosion can be performed by assessing the result of erosion, like the degree of softening or roughening of the early eroded surface using nano- or micro-hardness techniques, or in more advanced erosion, by measuring the amount of tooth structure loss [Stenhagen et al., 2010]. The use of these techniques is still largely limited to extra-oral assessments hence the study designs of investigations using these techniques are restricted to in vitro or in situ.

Results from laboratory studies are useful in determining proof of concept for both diagnostic systems and therapeutic interventions but rarely provide data that can be directly applied to the clinical situation. The techniques described above are designed for flat and polished samples, making the assessment of natural curved enamel with an intact fluoridated, aprismatic layer difficult if not impossible. In addition, dental erosion,
like dental caries is a multifactorial condition that involves the interplay between biological, chemical and behavioural factors [Lussi, 2006]. Even the most intricately designed in situ study fails to fully emulate the clinical environment, especially the interaction between the erosive and/or protective agent with saliva and the pellicle. Hence evaluation techniques that can be applied in an in vivo setting could yield additional information that is currently scarce in the literature.

5.2.2 In vivo assessment of early erosion lesion

An ideal in vivo assessment technique has to satisfy a few important criteria. Firstly, in order for an erosion assessment technique to be ethically employed in a clinical trial setting, it has to be able to detect erosion at its earliest stages before surface bulk loss occurs and when remineralisation of the lesion is still possible. The ability of a technique to detect early erosion lesions would also result in shorter clinical trial durations and therefore significantly reduce costs and probably increase recruitment and retention of subjects. The technique also has to be non-invasive and at the same time have the ability to monitor intervention-related lesion progression or regression over time. It should also enable repeated measurements to be taken quickly and with high precision.

In the early stages of dental erosion, the dissolution of hydroxyapatite prisms takes place within the prism sheath area and then spreads to the prism core. [Meurman et al., 1991; Meurman and Frank, 1991]. Eventually, fresh and unionised acid will then diffuse into the interprismatic areas of enamel [Featherstone and Rodgers, 1981] and further dissolve mineral in the subsurface region. This process results in a honeycomb pattern of prisms outlined by prism boundaries and has been demonstrated in many studies [Eisenburger et al., 2001; Lussi and Hellwig, 2001; Zheng et al., 2009]. This mineral loss results in porosity that extends a few micrometers below the surface and is known as softening [Koulourides, 1968]. The optical scattering properties of the enamel surface are altered due to this porosity and a strong correlation between mineral loss
and the scattering coefficient has been demonstrated in earlier studies [Brinkman et al., 1988; Darling et al., 2006]. These changes in optical scattering properties can be detected and quantified by optical systems and two of which that have potential to be used in vivo are Quantitative Light-Induced Fluorescence (QLF) and Optical Coherence Tomography (OCT).

5.2.3 QLF

Fluorescence results from a change in the wavelength of incident light following absorption and excitation of fluorophores in a material. In sound enamel, fluorescence is visible due to the fact that light is absorbed by and excites fluorophores in dentine, especially at the DEJ. In the presence of demineralisation, less fluorescence is observed. It has been suggested that scattering due to demineralisation causes less penetration of light into enamel and therefore lesser chance for photon absorption and fluorescence of the fluorophores in dentine [van der Veen and de Josselin de Jong, 2000]. Other researchers [Hafstrom-Bjorkman et al., 1992; Sundstrom et al., 1985] postulated that loss of fluorescence could be attributed to the loss of fluorophores during demineralisation. Earlier work on fluorescence utilised an argon-ion laser light source with wavelength of 488 nm but this was later replaced by regular white light source filtered with a blue band pass filter at approximately 370nm.

Much has been done to determine the relationship between the intensity of fluorescence and the mineral content of enamel. In vitro comparisons of fluorescence intensity with longitudinal microradiography (LMR) have been performed on artificial caries lesions [al-Khateeb et al., 1997; Hafstrom-Bjorkman et al., 1992], natural incipient caries lesions [Emami et al., 1996] and advanced enamel erosion [Pretty et al., 2004]. Strong correlations between amount of mineral loss and the amount of fluorescence loss have been found in all of the above studies with correlation coefficients ranging from 0.73 – 0.97. QLF can therefore be used as a non-destructive method to monitor the demineralisation and remineralisation processes longitudinally.
QLF has been used in caries clinical trials to evaluate the effectiveness of tooth brushing, dentifrices and fluoride varnishes on reducing the progress of caries [Al-Khateeb et al., 1998; Tranaeus et al., 2001]. Clinically, high repeatability and reproducibility of the QLF method was reported [Tranaeus et al., 2002].

When applied to enamel erosion, the use of QLF is less well described and its results contradictory for advanced erosive lesions when surface loss had occurred. Pretty et al [Pretty et al., 2004] in an *in vitro* longitudinal study, eroded human premolar in 30 minutes interval up to 15 hours. A strong positive correlation ($R^2 = 0.91$) between relative mineral loss as measured with TMR and loss of fluorescence was demonstrated. However, Elton et al [Elton et al., 2009] in an *in vitro* cross sectional study, found poor correlation between mineral loss and loss of fluorescence. They eroded bovine incisors six hourly up to 36 hours and found that there was a poor correlation between mineral loss and intensity of fluorescence in advanced enamel erosion lesions. This poor correlation could be due the use of bovine enamel [Laurance-Young et al., 2011] and to the extreme advance erosion lesion that was produced in this study as the erosion interval was three times that of Pretty et al’s in 2004 [Pretty et al., 2004]. With increased lesion depth, the floor of the crater becomes nearer to the source of fluorescence (dentine). This creates greater possibility of incident light reaching the enamel-dentinal junction and dentin thereby masking the scattering effect of the demineralised enamel overlying it.

5.2.4 OCT

OCT is analogous to ultrasound imaging except that it uses light instead of sound waves. It uses broadband near infra-red (NIR) light to perform cross-sectional imaging by measuring the magnitude and echo time delay of backscattered light. The magnitude of the backscattered light can be obtained from its depth resolved intensity curves or A-scans. Low-coherence interferometry is used to remove selectively or gate out the component of backscattered signal that has undergone multiple scattering.
events and a high quality cross-sectional image can then be generated by performing multiple axial measurements of this echo time delay (A – scans) and scanning the incident optical beam transversely. This produces a two-dimensional data set or B-scan, which represents the optical backscattering in a cross-sectional plane through the tissue. Hence, not only can it be used to produce qualitative morphologic cross sectioned images of near-surface tissue structures, it can also be used quantitatively by measuring the changes in the intensity of the backscattered light from different depths of the tissue investigated.

Incident light used in OCT for the imaging of dental hard tissue takes advantage of the fact that enamel is highly transparent in the NIR spectrum. For any particular wavelength, the optical properties of biologic tissue can be described quantitatively by defining the absorption ($\mu_a$) and scattering coefficients ($\mu_s$) and the scattering phase function $\Phi (\cos (\theta))$. $\mu_a$ and $\mu_s$ represent the probability of the incident photons being absorbed or scattered and $\Phi (\cos (\theta))$ is a mathematical function that describes the directional nature of scattering [Wilson et al., 1987]. Light absorption by enamel is very weak in the NIR spectrum [Fried et al., 1995] and therefore the optical behaviour of enamel and dentine in NIR are dominated by the scattering properties.

Darling et al [Darling et al., 2006] hypothesised that there are two types of scatterer structures in enamel. The first types are small hexagonal hydroxyapatite crystals of less than 50nm in diameter and these produce Rayleigh-like isotropic distributions and are responsible for the large fraction of isotropic scattering from the tissue. The second types are the large enamel prisms (3-6 µm in diameter) that are a few microns in cross section. These prisms produce Mie-like scattering effects which are highly forward directed. They also postulated that demineralisation substantially increased the proportion of the isotropic (Rayleigh-like) scatterers. Hence, when light travels through demineralised enamel, not only is it scattered by the enamels rods, it is also scattered
by the multitude of pores resulting from demineralisation, resulting in greater intensity of the backscattered light and changes in the optical refractive index.

Previous qualitative assessment of demineralisation has mainly been performed for early enamel caries and the emphasis was on distinguishing between sound and early carious enamel [Everett et al., 1999]. Hewko et al [Hewko et al., 2005] had also explored and suggested coupling the use of Raman Spectroscopy with OCT where OCT was used as a rapid method to screen a relative larger region for a demineralised area and for the assessment of lesion depth, while the Raman probe is then used to provide biochemical investigation of the detected demineralised area.

The utility of the optical scattering measurements from OCT and polarisation-sensitive OCT (PS-OCT) in quantifying demineralisation has been validated by comparing it with relative mineral volume changes in TMR measurement [Darling et al., 2006; Fried et al., 2007] - a non-linear relationship between mineral loss and the scattering coefficient was demonstrated. There is a large increase in scattering with slight demineralisation, followed by minimal increase in scattering for the more severe lesions [Darling et al., 2006]. Longitudinal measurement of early artificial demineralisation suggested a similar rapid exponential increase in the backscattered intensity during initial lesion development, followed by a more gradual increase as the lesion severity increases [Huynh et al., 2004]. Amaechi et al [Amaechi et al., 2001] demonstrated that the loss of penetration depth in conventional OCT images correlated well with the mineral loss measured with microradiography for shallow artificial lesions on smooth surfaces.

The use of OCT has evolved from Colston et al’s [Colston et al., 1998] and Feldchtein et al’s [Feldchtein et al., 1998] initial description of in vivo acquisition of images of hard and soft tissue to its recent in vivo use to monitor progression of caries in orthodontic patients [Fried et al., 2010] and in a clinical trial to evaluate the efficacy of a proton-pump inhibitor to reduce erosion in patients with gastroesophageal reflux disease [Wilder-Smith et al., 2009].
Although similar demineralisation processes occurs in caries and erosion, the histology, localization and pattern of progression are different between the two. Caries is a subsurface phenomenon, while erosion is a superficial condition. Therefore, the presentation of the intensity of the backscattered light may be different. Little has been done to explore the use of OCT in detecting and quantifying erosion except for Wilder-Smith et al’s work with patients with gastroesophageal reflux disease, whose erosion rate were aggressive. There is scarce information as to how sensitive OCT is to small demineralisation changes.

Both OCT and QLF have the potential to being used in longitudinal erosion studies in vivo. Before employing these techniques in clinical settings, it is necessary to first establish in vitro the sensitivity of these techniques in quantifying demineralisation of early enamel erosion longitudinally. It is also important that the validation be undertaken on natural, unpolished enamel surfaces.

5.3 Objectives and Working Hypothesis

The objectives of this study were to determine:

I) Whether the progression of early enamel erosion can be detected using QLF and OCT in vitro.

II) The detection threshold of QLF and OCT for early enamel erosion lesions.

The Null Hypothesis tested was QLF and OCT is not able to detect the progression of early enamel erosion in vitro.
5.4 Materials and Methods

The current study model was designed to simulate the clinical production of the early stages of dental erosion, which involves surface-softening with no evidence of surface loss.

5.4.1 Sample preparation and erosion cycle

A convenient sampling of 12 extracted upper or lower human central incisors, caries free with no visually obvious fluorosis, were used in this study. The teeth were collected by the Kerala Dental School and were stored in thymolised water before the start of the study. Each tooth was fully embedded in separate cold cured methyl-methacrylate resin block with a dimension of 10 mm x 10 mm x 30 mm except for a 5 mm x 5 mm window at the middle third of its labial surface. The incisors were mounted such that the exposed labial surface was at least level with or not more than 1mm higher than the adjacent resin. This was to ensure optimum contact with the orange juice and to keep the height variation consistent with the depth of field of QLF. One half of the exposed labial surface was protected with a non-residue masking tape while the other half was being coated with a one-step self-etch adhesive, Xeno® V (DENTSPLY), according to the manufacturer's instruction. This adhesive coated area served as the reference area. After the application of the Xeno® V (DENTSPLY) was completed, the protecting masking tape was removed from the other half for exposure to the erosive challenge. After the application of the adhesive, baseline surface microhardness (SMH) measurements of the exposed area were recorded and baseline QLF and OCT images were taken of both the exposed and non-exposed areas.

The teeth mounted in the resin blocks were then suspended using plastic rods into a commercially available orange juice (ASDA Orange Juice from Concentrate) (pH 3.8 ± 0.1). Two groups of six teeth were suspended each in 500 ml of orange juice and the orange juice was gently stirred with a magnetic stirrer. The pH of the orange juice was monitored with a pH meter throughout the erosion cycle. After every 10 minutes for up
to a total of 60 minutes, the teeth were removed from the orange juice and rinsed under a reservoir of running water for 1 minute to remove excess acid from the surface of the teeth. The samples were then dried for 20 seconds with compressed air that is fixed 10cm away from the resin block and measurements taken with all three instruments. The orange juice was changed after every 10 minutes of erosive challenge interval.

5.4.2 Surface microhardness

The micro-indenter, Microhardness Tester FM-700; Future-Tech Corporation, Japan was used in the study. The resin blocks were placed flat on the translation stage and fixed at a reproducible position with the precision vice of the micro-indenter. A surface area of approximately 1 mm x 1 mm perpendicular to the direction of the load of the indenter was identified in the uncoated half of the enamel window for indentation. The identification of the perpendicular area was done by ensuring that the aiming beam of the micro-indenter was in focus before the loading was performed. Micro-indents were made using a Knoop diamond indenter, with a load of 25 g applied for 5 seconds.

Five indentations approximately 100µm apart were made during each measurement time point at the identified measurement area. The horizontal lengths were measured and the Knoop numbers \(KHN(t)\) were calculated and averaged. Although care was taken to ensure that indentations were done on a surface that was perpendicular to the direction of loading, due to the inherent curvature of a natural and unpolished enamel surface and the presence of local irregularities such as perikymata, some asymmetrical indenters were observed and were not included in the analysis. In these cases, additional indenters were made. The outcome measure was expressed as the percentage of surface microhardness change \(\Delta SMC\), calculated based on the differences between Knoop hardness numbers at baseline, \(KHN(t_0)\) and the subsequent erosion intervals, \(KHN(t)\).

\[
\Delta SMC(t) = 100 \left[ \frac{KHN(t) - KHN(t_0)}{KHN(t_0)} \right]
\]
5.4.3 QLF

An in-house developed QLF set up was built. Illumination was based on a custom-made 395 nm-LED array (B5-437-CVD, Roithner Lasertechnik GmbH, Austria), designed in a ring configuration around the imaging lens to ensure uniform illumination over the sample. A blue band pass filter centred at 425 nm, was fitted in front of the array to prevent any light above 450 nm emitted from the LED reaching the sample. Images were captured with a triple charged coupled device (3CCD)-colour camera of 1014 x 768 pixel resolution (HV-F31, Hitachi Kukasai Electric U.K. Ltd.) A 50 mm focal length-imaging lens (Fujinon HF50HA-1B, Fujifilm U.K. Ltd.) with a 10 mm-extension was attached to the camera and a 515 nm long-pass yellow filter (OG-515, Edmund Optics Ltd, U.K.) was used to filter the fluorescence light emitted by the sample.

The images were taken in a dark enclosure and captured with bespoke software which enabled repositioning of the samples, using the video camera, at the various measuring time points with respect to baseline. An outline of the baseline image in the form of an overlay was superimposed on the live video of the image to facilitate alignment before an image was captured. Optimum light intensities and camera settings were determined to ensure that the fluorescence images from the sample were within the detection dynamic range of the camera and of maximum contrast.

A program was written in MATLAB (The MathWorks Inc, USA) to analyse the images. Images from each specimen at the various erosion intervals were aligned so that representative regions of interest (ROI) of the same location and dimensions throughout the erosion interval points for the exposed and non-exposed areas could be drawn.

The mean pixel value of the green channel was obtained for the defined ROI for the exposed, $F_E(t)$, and non-exposed areas, $F_{NE}(t)$. All images were analysed blind to the erosive challenge intervals, $t$. The percentage loss of fluorescence of the exposed area at each erosion interval, $\Delta F(t)$, was calculated as follows:

\[ \Delta F(t) = \frac{F_{NE}(t) - F_E(t)}{F_E(t)} \times 100 \]
\[ \Delta F(t) = 100 \left[ \frac{F_E(t)}{F_E(t_0)} - \frac{F_{NE}(t)}{F_{NE}(t_0)} \right] \]

where \( t_0 \) is the baseline time point.

### 5.4.4 OCT
A commercially available OCT system (OCS1300SS, Thorlabs Ltd, UK) was used to capture cross sectional images of the exposed window of the tooth surface. The OCS1300SS uses Fourier Domain technology and incorporates a broadband, frequency swept laser centred at 1325 µm. It has an axial resolution of 9 µm and transverse resolution of 15 µm in air according to the manufacturer. The hand probe was mounted with the beam facing downwards. The samples were placed on a translational stage perpendicular to the hand probe. The stage was fixed with a repositioning jig that enabled the sample to be repositioned in the same position and alignment at the different measuring time points.

The Thorlabs Swept Source OCT software was used for image capturing and for the control of the OCT settings and light beam. The light beam was configured to scan a length of 5mm in the mesio-distal direction (x-axis) of the labial surface of the sample (Figure 5.1) and at an axial depth of 3 mm. The inciso-cervical position of the light beam on the tooth surface (y-axis) was located at a cross section with the least observed specular reflection. The \((x, y)\) coordinate of the light beam for each sample was recorded for replication at consecutive measuring time points. The distance of the tooth surface to the probe was determined with the most convex area of the labial surface of the tooth at 1.0 ± 0.1 mm from the top of the display window of the image capture software. The maximum amplitude was calibrated with an eroded enamel sample at the beginning of each study day and the dynamic range of the OCT light was maintained to be around 20 – 30 decibels (dB), as suggested by Baumgartner et al [Baumgartner et al., 2000]. Background noise was removed before the acquisition of each image [Schoenenberger et al., 1998].
A program was written in MATLAB (The MathWorks Inc, USA) to analyse the changes of the OCT backscattered light intensity in time. The B-scans of each sample from the different measuring time points were aligned and a similar region of interest was selected for all seven measuring time points. This region of interest consisted of 150 A-scans in each of the exposed and non-exposed areas, as shown in Figure 5.2. The curvature of the tooth surface in the selected region of interest was compensated by aligning the peak of the backscattered intensity rise occurring at the enamel-air interface in each A-scan along the same horizontal pixel line of the B-scan. A mean A-scan was then generated from the selected 150 A-scans for both the exposed and non-exposed area.

The outcome measure for OCT was expressed as the mean percentage difference of decay, $\Delta D$, between the exposed and non-exposed area. The decay, $D$, is the relationship of the attenuation of backscattered light between two optical depths of an OCT A-Scan and is represented by the function below,

$$D = \frac{I_{\text{plateau}}}{I_{\text{superficial}}}$$

$I_{\text{superficial}}$ is the intensity of backscattered light at or immediately below the tooth-air interface where demineralisation had occurred. The chosen superficial optical depths for this study were 0 µm (tooth-air interface), 10 µm, 20 µm, 30 µm, 40 µm and 50 µm below the tooth-air interface (Figure 5.3). With the refractive index of enamel being 1.62, these chosen levels translated to the physical depth of 0 µm, 6.25 µm, 12.5 µm, 18.75 µm, 25 µm and 31.25 µm below the tooth-air interface respectively. $I_{\text{plateau}}$ is the intensity of the backscattered light where the A-scans had reached a plateau and were assumed not affected by the erosive challenge. It was observed to be at the optical depth of 150 µm for this study.
\( \Delta D(t) \) at each measurement time point, \( t \), is represented by the function below:

\[
\Delta D(t) = 100 \left[ \frac{D_E(t)}{D_E(t_0)} - \frac{D_{NE}(t)}{D_{NE}(t_0)} \right]
\]

where \( D_E \) is \( D \) of the exposed area, \( D_{NE} \) is \( D \) of the non-exposed area, \( t_0 \) is the baseline time point and \( t \) is the erosive challenge time point.

### 5.4.5 Statistical Analysis

The STATA™ 10.1 (Statacorp, Texas) statistical program was used. Multiple linear regression analyses were performed, taking into account the clustering of samples using robust variance estimates, to ascertain whether time related changes were detected with the three instruments.

When a significant time-related change was found for an outcome measure, paired t-test was performed to identify the detection sensitivity, which is defined as the shortest subsequent time interval when a significant difference was detected.
5.5 Results

5.5.1 Surface Microhardness

Multiple regression analysis of $\Delta SM$ with erosion interval showed that surface microhardness detected a significant erosion-interval related decrease in microhardness with a mean of about 80% at the end of the 60 minutes of erosion. A linear function could be fitted to it with a $R^2$ value of 0.810 ($P < 0.05$). The graph in Figure 5.4 shows the mean sample $\Delta SM$ for each erosion interval.

Paired t-tests were used to analyse $\Delta SM$ between each consecutive interval of erosion. Significant decreases in surface microhardness ($P<0.05$) were detected between every 10 minutes of erosive challenge. There was approximately a 40% decrease in surface microhardness for the first two erosion intervals but a tapering of the decrease was found thereafter. At the 10-minute erosion time point, the $\Delta SM$ from baseline was 23.8 +/- 5.5 % (mean +/- standard error (SE)) ($P=0.001$) while at 60 minutes of erosion, the percentage change of the surface microhardness from the preceding erosion interval was 5.64 +/- 1.9 % (mean +/- SE) ($P=0.007$) (Table 5.1).

5.5.2 QLF

Multiple regression analysis of $\Delta F$ with erosion interval showed that QLF detected significant erosion-interval related loss of fluorescence. A linear function could be fitted to it with an $R^2$ value of 0.590 ($P < 0.05$). The graph in Figure 5.5 shows the mean loss of fluorescence with erosion interval. Figure 5.6 presents the QLF images of a sample showing the increased loss of fluorescence of the exposed area as compared to the non-exposed area with the progression of erosion. It was found that there was 1.28 +/- 0.55 % (mean +/- SE) loss of fluorescence during the first 10 minutes of erosion and cumulatively 8.90 +/- 0.83 % (mean +/- SE) fluorescence loss at the end of 60 minutes of erosion.
\( \Delta F \) and \( \Delta SMC \) values were plotted against each other in a scatter plot and a regression line calculated as shown in Figure 5.7. A significant positive Spearman’s rank correlation factor of 0.727 (P < 0.05) between \( \Delta F \) and \( \Delta SMC \) was observed.

Paired t-tests were used to analyse the differences of the loss of fluorescence between each consecutive interval of erosion. Significant differences (P<0.05) were first detected between baseline and 10 minutes erosion and thereafter every consecutive 20 minutes of erosive challenge (10 – 30 minutes, 30 – 50 minutes) and also between the last 10 minutes of erosion (Table 5.1).

### 5.5.3 OCT

Multiple linear regression analysis showed that there were significant erosion-interval related changes in \( \Delta D \) for the depth combinations of 150 µm/20 µm, 150 µm/30 µm, 150 µm/40 µm and 150 µm/50 µm (P <0.05) but not for 150 µm/0 µm and 150 µm/10 µm. \( \Delta D \) (150 µm/50 µm) demonstrated the highest R-square value of 0.319 and followed by \( \Delta D \) (150 µm/30 µm) with a value of 0.312 (Table 5.2).

\( \Delta D \) (150 µm/30 µm) was plotted against \( \Delta SMC \) in a scatter plot and a regression line calculated as shown in Figure 5.8. A significant (P < 0.05) positive Spearman’s rank correlation factor of 0.56 was found.

Figure 5.9 illustrates the progression of \( \Delta D \) throughout the erosive challenge for the various depth combinations that showed significant time related changes. \( \Delta D \) for the depth combinations of 150 µm/20 µm, 150 µm/30 µm, 150 µm/40 µm and 150 µm/50 µm demonstrated a gradual decrease as erosive challenge progresses and at the end of the 60 minutes erosive challenge, \( \Delta D \) for these depth combinations decreased 30 - 40 %. Half of this increase occurred early, during the first 10 minutes for the depth combination of 150 µm/20 µm and 150 µm/30 µm while the main decrease occurred between 10 and 20 minutes for the depth combination of 150 µm/40 µm and 150 µm/50 µm.
μm. \( \Delta D \) for 150 μm/10 μm and 150 μm/0 μm on the other hand did not show any obvious trend with erosive challenge.

Paired t-tests were also performed to evaluate the detection sensitivity of these four optical depth combinations and the results are presented in Table 5.1. The \( \Delta D \) for depth combinations of 150 μm/30 μm and 150 μm/40 μm both showed significant difference in decay at three time points. \( \Delta D \) of 150 μm/30 μm demonstrated early detection sensitivity at 10 minutes but subsequently only at 50 minutes and 60 minutes of erosive challenge. \( \Delta D \) for 150 μm/40 μm demonstrated significant difference in decay at every 20 minutes interval. 150 μm/20 μm and 150 μm/50 μm showed significant difference in decay at only two time points and of longer intervals, as shown in Table 5.1.
5.6 Discussion

The current study model was designed to simulate the clinical stages of early enamel erosion. The degree of demineralisation incurred in the present study was small, only involving surface-softening with no evidence of surface loss or step change of more than 10 microns as observed in the B-Scans of OCT at the end of the erosion interval (Figure 5.10).

All the outcome measures except were expressed in percentages of the non-exposed area, to account for biological variations between different teeth and systematic instrumental variation. The regression analyses were performed as linear analyses as there were only 6 separate measurements available.

5.6.1 Surface Microhardness

Featherstone et al [Featherstone et al., 1983] using an artificial caries model demonstrated a direct relationship between volume percent mineral and the square root of the Knoop hardness values for 40 – 90% demineralised dental enamel and concluded that although microhardness and microradiographic profiles measure different physical properties, the two are closely interlinked and microhardness profiles therefore be used not only as a comparative measure of hardness changes but as a direct measure of mineral loss as a consequence of demineralisation. Hara and Zero [Hara and Zero, 2008] and Jaeggi and Lussi [Jaeggi and Lussi, 1999] have also shown that surface microhardness is sensitive for detecting the initial stages of erosion with softening of the enamel surface but has limitations in the analysis of advanced lesions with substance loss. Therefore surface microhardness was chosen as the established assessment technique for early erosion with which QLF and OCT were compared in this study.

The use of flat, polished surfaces has been recommended to produce well-defined indentations but in order to satisfy the objective of this study of validating the use of QLF and OCT for the end purpose of application in clinical trials, natural, unpolished
labial surfaces were used. Caldwell et al [Caldwell et al., 1957] first described the measurement of surface microhardness on intact surface of enamel. They found that after excluding asymmetrical indentations, the variation in hardness of intact surfaces is usually the result of local differences in hardness of the intact surfaces and not attributable solely to mechanical difficulties in obtaining symmetrical indentations.

The labial surface of upper and lower central incisors were chosen for this study due to their relatively flat profile. The mean KHN for this study ranged from 306.5 ± 24.8 at baseline to 54.44 ± 7.67 after 60 minutes of erosive challenge in orange juice. The standard deviations of the indentations measured were comparable to those of polished flat surfaces [Marillac et al., 2008].

In order to limit the impact of surrounding material changes, Featherstone et al and Lussi et al [Featherstone et al., 1983; Lussi et al., 1995] suggested that micro indentations are to be performed with a low pressure of not more than 50g. The load of 25g with a dwelling time of 5s was chosen for this study as it was the smallest load with the shortest loading time needed to produce a detectable indent under 50x magnification for all the 12 samples at baseline. This was especially important when specimens with natural surface were used. To start with the smallest detectable indent would mean it was less likely to result in with large indents that surpassed the dimensions of a flat plane present on the slightly curved surface of a central incisor.

Although linear regression analysis was performed, as there were only 6 separate measurements available, the graph of the mean ΔSMC with erosion interval (Figure 5.4) showed a trend of exponential decrease in of ΔSMC with erosion interval rather than a linear one and is similar to that found in previous studies on polished samples [Lippert et al., 2004; Zheng et al., 2009].

5.6.2 QLF

A customized QLF set-up was used in this study in an attempt to increase the sensitivity of the system by optimizing the illumination and increasing the resolution of
the image acquired. The levels of fluorescence loss measured with a specific QLF camera are influenced by various system specifications of the set-up [Lagerweij et al., 1999] and type of substrate, hence the level of fluorescence loss in this study could not be directly compared with other QLF studies. However, the level of fluorescence loss of this study is similar to that of Ablal et al’s [Ablal et al., 2009] and Pretty et al’s [Pretty et al., 2004], despite different types of samples and QLF system used. Ablal et al subjected polished bovine incisors to orange juice and from figure 1 of their article, there was approximately a 2% loss of fluorescence at 20 minutes of erosive challenge with orange juice. Pretty et al on the other hand demonstrated approximately a 2% loss of fluorescence at 30 minutes of erosive challenge with 0.1% citric acid on natural surface of extracted human premolars. As shown in Figure 5.5 of this study, there was 3.14 +/- 0.91 % (mean +/- SE) of mean fluorescence loss after 20 minutes of erosive challenge with orange juice.

5.6.3 OCT

At the enamel-air interface, specular reflection is very strong and it could be around 20dB higher than the backscattering intensity. This can therefore mask any information about scattering at or just below the tooth surface [Fried et al., 2002]. This strong specular signal can lead to residual coherence artefacts or spikes in the image. The B-scan of a sample as shown in Figure 5.11 illustrates the ‘columnar’ artefacts resulting from specular reflection. Amaechi et al [Amaechi et al., 2001] in their attempt to minimize the confounding effect of specular reflection during the quantification of early artificial caries described the exclusion of a variety of distances from the surface for different specimens when identifying as cut-off depths. As the pattern of demineralization of erosion lesions are generally more homogenous and uniform than caries and with \( \Delta D \) of the various combination depths, a more systematic way of ascertaining a cut-off level for exclusion of the effect of specular reflection is possible.
With the combination depth of 150 µm/0 µm and 150 µm/10 µm, no time-related changes of ΔD were observed throughout the 60 minutes of erosion intervals. This is most likely due to the backscattering signal being confounded or masked by the much stronger specular reflection.

Although the linear regression results of ΔD (150 µm/20 µm) showed significant erosive challenge related changes and a gradual decrease of mean percentage difference in decay from baseline to 40 minutes of erosion was observed, there was a sudden increase of mean percentage difference in decay at 50 minutes of erosion (Figure 5.9), suggesting that the backscattered intensity at 20 µm might still be confounded by surface reflectance signals, though at a lesser magnitude. Therefore the statistical cut-off depth for specular reflection for this study is 20 µm.

The depth combination of 150 µm/30 µm and 150 µm/40 µm seemed to be the optimum depths to be used in the measuring of early demineralisation with OCT. These two depth combinations are the levels nearest to the tooth-air interface that showed significant linear regression with erosion interval but were not affected by specular reflection. Both combinations also demonstrated significant difference in decay at three time points. For a small erosive challenge, the ΔD at 150 µm/30 µm seemed to be the combination depth of choice as it detected a significant difference as early as 10 minutes of erosion but during the subsequent erosion challenges, it was not as sensitive as ΔD of 150 µm/40 µm. ΔD of 150 µm/40 µm detected significant difference uniformly at every 20 minutes of erosive challenge throughout the 60 minutes of erosive challenge, hence is the preferred combination depth to be considered in the current study design.

In this study, it was found that there were significant positive correlations between the outcome measure of both QLF and OCT with erosion interval and surface microhardness. Nevertheless, within the limits of the current design, QLF seemed to be a better diagnostic tool than OCT in the detection and monitoring of demineralisation,
as it demonstrated a higher correlation with erosion interval and surface microhardness. It also demonstrated better detection sensitivity than OCT ($\Delta D$ of 150 $\mu$m/40 $\mu$m), as presented in Table 5.1.

OCT, however, provides additional information about the subsurface characteristics of the eroded enamel. It not only provides information about the degree of demineralization from the backscattered intensity, it also provides information about lesion depth. As demineralisation progressed, the progression of porosity from the enamel-air interface towards the deeper layers is indirectly mirrored by the chronological progression of the occurrence of significance of difference in decay from the more superficial layers of enamel to the deeper layers (Figure 5.9). This feature could potentially be used to compare the severity of erosion between samples at one particular time point or in a cross sectional study design. The OCT backscattered signal is also not confounded by discolouration – an issue that frequently affects QLF. A discoloured area viewed with light-induced fluorescence would appear dark like a demineralised area. This is due to absorption of light in the discoloured area [van der Veen and de Josselin de Jong, 2000].

The lower correlation of OCT with erosion interval and surface microhardness could be attributed to the larger standard deviations of the OCT measurements. These larger standard deviations could be due to a combination of deviations in repositioning between the various measurement time points and residual effects of specular reflection. In this study the OCT measurements are more susceptible to deviations in repositioning as only one cross-section (B-Scan) was acquired while the QLF measurements were from an area. The problems encountered with OCT could potentially be minimised by taking three dimensional images and / or using polarisation-sensitive OCT.
5.7 Conclusion

1. Both QLF and OCT were able to detect the progression of *in vitro* early enamel erosion.

2. The *in vitro* early enamel demineralisation detection threshold of the QLF set up in this study is 10 minutes of erosive challenge with orange juice.

3. The *in vitro* early enamel demineralisation detection threshold of the OCT set up in this study varies with the different depth combination. For $\Delta D$ (150 µm/30 µm), it is 10 minutes of erosive challenge with orange juice.
5.8 References


## 5.9 Tables

Table 5.1
Detection Sensitivity of Surface Microhardness, QLF and OCT

<table>
<thead>
<tr>
<th>Outcome Measure</th>
<th>10 minutes</th>
<th>20 minutes</th>
<th>30 minutes</th>
<th>40 minutes</th>
<th>50 minutes</th>
<th>60 minutes</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\Delta SMC$</td>
<td>P=0.001</td>
<td>P=0.008</td>
<td>P=0.016</td>
<td>P=0.006</td>
<td>P=0.017</td>
<td>P=0.007</td>
</tr>
<tr>
<td>$\Delta F$</td>
<td>P=0.023</td>
<td>P=0.000</td>
<td>P=0.013</td>
<td>P=0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\Delta D$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(150µm/20µm)</td>
<td>P=0.023</td>
<td></td>
<td>P=0.024</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(150µm/30µm)</td>
<td>P=0.005</td>
<td></td>
<td>P=0.001</td>
<td>P=0.049</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(150µm/40µm)</td>
<td>P=0.026</td>
<td></td>
<td>P=0.034</td>
<td>P=0.028</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(150µm/50µm)</td>
<td>P=0.005</td>
<td></td>
<td></td>
<td>P=0.029</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table shows the result of paired t-test between each consecutive interval of erosion for $\Delta D$ of various depth combinations, $\Delta SMC$, and $\Delta F$. Only changes that were significantly different (P<0.05) were recorded in this table.
Table 5.2

Result of Multiple Linear Regression Analysis of $\Delta D$ with Erosive Challenge Interval

<table>
<thead>
<tr>
<th>Superficial optical depth (µm)</th>
<th>Plateau optical depth (µm)</th>
<th>$R^2$</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>150</td>
<td>0.021</td>
<td>0.195</td>
</tr>
<tr>
<td>10</td>
<td>150</td>
<td>0.040</td>
<td>0.101</td>
</tr>
<tr>
<td>20</td>
<td>150</td>
<td>0.172</td>
<td>0.001</td>
</tr>
<tr>
<td>30</td>
<td>150</td>
<td>0.312</td>
<td>&lt; 0.000</td>
</tr>
<tr>
<td>40</td>
<td>150</td>
<td>0.305</td>
<td>&lt; 0.000</td>
</tr>
<tr>
<td>50</td>
<td>150</td>
<td>0.319</td>
<td>&lt; 0.000</td>
</tr>
</tbody>
</table>

Table shows multiple linear regression analysis of $\Delta D$ for all 6 depth combinations with erosion interval. $\Delta D$ (150 µm/20 µm), $\Delta D$ (150 µm/30 µm), $\Delta D$ (150 µm/40 µm) and $\Delta D$ (150 µm/50 µm) showed significant erosion-related changes while $\Delta D$ (150 µm/0 µm), $\Delta D$ (150 µm/10 µm) did not.
5.10 Figures

Figure 5.1

The labial surface of a specimen with the red aiming beam showing the position of the chosen OCT cross section
**Figure 5.2**

The upper half of the figure is the B-Scan of a specimen recorded after 30 minutes of erosion. Patch (i) and (ii) are areas with 150 A-scans of interest at the non-exposed and exposed sides. The bottom half of the figure show the mean A-scans that were generated from the selected 150 A-scans from the non-exposed (blue line) and exposed side (red line).
Figure 5.3

The figure shows mean A-scans of the exposed side of a sample at baseline, 20, 30 and 50 minutes of erosive challenge. The optical depths of $I_{\text{superficial}}$ chosen for the study were 0, 10, 20, 30, 40 and 50µm from the tooth-air interface. The observed optical depth for $I_{\text{plateau}}$ where the backscattered intensity reached a plateau was 150µm.
Figure 5.4

Line graph showing the decrease of percentage change of surface microhardness ($\Delta$SMC) as the erosion interval progressed.
Figure 5.5

Line graph showing the mean percentage loss of fluorescence ($\Delta F$) as the erosion interval progressed.
**Figure 5.6**

QLF images of a sample at baseline and at the end of the 60 minutes of erosive challenge. E—Exposed side, NE—Non-exposed side. There is increased loss of fluorescence of the non-coated side as erosion progressed.
Figure 5.7

Scatterplot of the validation of QLF by surface microhardness.

The Spearman rank correlation factor is bigger than that demonstrated by OCT (Figure 5.7) It is observed that one specimen labelled with the sign + could be an outlier showing values that were far from the mean.
Figure 5.8

Scatterplot of the validation of OCT ($\Delta D$) by surface microhardness. The Spearman's rank correlation factor is less than that of QLF (Figure 5.7) with more specimens showing values away from the mean.
Figure 5.9

Mean percentage difference of decay of backscattered intensity between exposed and non-exposed areas, (ΔD), for the depth combinations of 150 µm/20 µm, 150 µm/30 µm, 150 µm/40 µm and 150 µm/50 µm as the erosion interval progressed.
Figure 5.10

The OCT B-Scan of a sample after 60 minutes of erosive challenge. The arrow points to the border of the exposed and non-exposed surfaces. There is no discernible step change between the two surfaces (Based on the axial resolution of the OCT of 15µm).
Figure 5.11

OCT B-scan of a sample with specular reflection observed at the tooth-air interface and resulting 'column' artefact below the surface.
CHAPTER 6

MEASURING EARLY ENAMEL EROSION WITH OCT & QLF: A STUDY OF REFERENCE METHODS
6.1 Abstract

**Background:** As shown in Chapter 5, early enamel demineralisation caused by erosion can be quantified *in vitro* using OCT and QLF. Like other techniques used for monitoring changes in demineralisation over time such as surface profilometry, a reference area not affected by the erosive challenge is needed to account for systematic errors and biological variability.

**Objective:** The objective of this study was to monitor the progression of demineralisation in early eroded enamel with OCT and QLF using two different reference methods which could be applied *in vivo*. The two reference methods were eroded enamel moistened with water and sound enamel coated with a dentine bonding agent.

**Methods:** A convenient sampling of 12 human incisors were embedded in resin except for a 5 mm x 5 mm window at the labial surface. Half of the window was coated with a self-etching dentine bonding agent, Xeno® V (DENTSPLY) while the other half was left exposed. Baseline measurements were taken with OCT and QLF. A commercially available OCT system (OCS1300SS, Thorlabs Ltd, UK) was used to capture cross sectional images of the window. The intensity of backscattered light from the various optical depths of the exposed and reference areas were analysed. An in-house developed QLF set up with a 3CCD colour camera, blue LEDs in a ring illuminator and a long-pass yellow filter was used. The loss of fluorescence with time was compared between the exposed and reference areas. Specimens were immersed in orange juice (pH 3.8 ± 0.1) in 10 minute-stepwise periods of up to 60 minutes. After every 10 minutes, the teeth samples were rinsed with water, dried and measurement taken of the exposed and reference areas. The exposed area was then remoistened with water and another measurement was taken. Multiple linear regression and paired t-test were used to compare the change of the outcome measures at the different erosive challenge intervals with respect to baseline.
**Results:** For QLF, multiple linear regression of both reference methods showed a significant dose response with erosion interval. The non-exposed surface technique showed significant mean percentage loss of fluorescence (P<0.05) from baseline after 10 minutes of erosive challenge (1.33 ± 0.50 (mean ± SE)), while the moistened exposed surface technique showed a significant loss at 30 minutes, (5.47 ± 2.60 (mean ± SE)).

For OCT, multiple linear regressions of both reference methods also showed significant dose response but the non-exposed surface technique showed a much stronger relationship than the moistened-exposed technique. With the depth combination of 150 µm/30 µm, both reference techniques showed significant percentage difference of decay (P<0.05) from baseline after 10 minutes of erosive challenge but the moistened-surface technique did not show any more significant difference in decay thereafter while the difference of decay for the non-exposed surface technique continued to be significant up to end of the study.

**Conclusion:** It is concluded that both reference methods can be used to reference in vitro longitudinal erosion measurements but in the present study design, the non-exposed surface technique is more sensitive than the moistened-surface technique for the detection of changes in early demineralisation.
6.2 Introduction

Dental erosion is defined as a chemical process that involves the dissolution of enamel and dentine by acid not derived from bacteria [Larsen, 1990]. It initially results in the softening of enamel due to the increase in porosity of the tooth. This causes a change in its optical scattering properties and attempts have been made to quantify these changes with OCT and QLF [Amaechi et al., 2003a; Amaechi et al., 2003b; Popescu et al., 2008; Pretty et al., 2004a].

OCT performs cross-sectional imaging by measuring the magnitude and echo time delay of backscattered light from a tissue. It enables non-invasive 'optical biopsies' to be taken from a tissue by illuminating it with a broadband light source. The intensity of the reflected light can then be measured from the resulting A-scans and also recorded for image processing by generating B-scans.

QLF is based on the fluorescence of enamel and dentine. The devise measures differences in this fluorescence related to mineralisation levels in enamel. As previously shown in chapter 5, QLF and OCT may be used to quantify early dental erosion in vitro.

The testing of the efficacy of preventive measures often involves longitudinal monitoring of the progression of demineralisation where it is essential that the same region of interest is being measured at different time points. Therefore the instrument used for measurement should ideally be non-destructive to the surface of interest and at the same time enable the performance of repeated measurements quickly and accurately. The technique should also ensure optimal repositioning of the sample during the various measuring time point.

6.2.1 Need for a reference area

Like other techniques used for monitoring demineralisation changes over time, a reference area not affected by the erosive challenge is needed to account for systematic errors and biological variability. In general, an ideal reference technique
would be one that is able to rely on a reference area that is resistant to the acid challenge, stays constant throughout the period of the study and is not technique - or operator - sensitive. In addition to that, specifically in relation to the QLF system, it should also be resistant to staining. In order for a technique to be appropriate for application in vivo, it should also be non – invasive, biocompatible and bio-inert.

The reference method most frequently used in previous in vitro and in situ erosion studies is coating an area of the enamel or dentine samples with acid-resistant nail varnish. It is inappropriate to use nail varnish on teeth in vivo. Dentine adhesive agents [Gernhardt et al., 2007; Sundaram et al., 2007] or dental varnishes [Kato et al., 2009; Murakami et al., 2009] have been proven to inhibit demineralisation of enamel and dentine affected by extrinsic acid like acidic beverages, therefore could potentially be used instead of nail varnishes. Alternatively it might be possible to apply an index-matching solution that fills the porosity of the demineralised surface and reproduce the refractive index of sound enamel.

6.2.2 Dentine bonding agent

Dentine bonding agents are normally bisphenol-A–glycidyl methacrylate (bis-GMA) based resin. The basic mechanism of its bonding to enamel and dentine is essentially an exchange process involving replacement of minerals with the resin, which upon polymerisation become micro-mechanically interlocked with the hard tissue [Buonocore et al., 1968; Gwinnett and Matsui, 1967].

Although commonly used in conjunction with dental composite for the restoration of carious enamel or dentine, dentine bonding agents have also been recommended to be used alone for dentine hypersensitivity and protection of exposed root. Gernhardt et al [Gernhardt et al., 2007] found that demineralization on root dentine was inhibited when it was coated with dentine bonding agents. They have also been recommended as a management method to reduce the rate of erosive wear of dentine [Sundaram et al., 2004].
6.2.3 Index-Matching Agent

The refractive index of sound enamel in visible light is 1.62 and under the near infra-red (NIR) spectrum it is 1.631 [Meng et al., 2009], while the refractive index of water is 1.33 in visible light and 1.32 under near infrared. Sound enamel consists mainly of carbonate-rich and calcium deficient hydroxyapatite crystals which are very densely packed, giving it a glass-like, translucent appearance. Water is partly bound to the organic matrix and forms the hydration layer of apatite crystallites and is partly present in a freely mobile form in the tail region of the prisms and interprismatic regions [Little and Casciani, 1966].

During demineralisation *in vivo*, minerals along with the bound water are lost and if in a moist environment, are replaced by water. Water has a refractive index (RI) nearer to enamel than air and hence when the spaces previously occupied by minerals are filled with water rather than air, the refractive index and the scattering properties of the moistened demineralised enamel is closer to that of sound enamel than when it is dry. Water penetrates even very small pores in enamel because of its small molecular size and it is compatible with the mineral and protein components of enamel. When water evaporates from demineralised enamel, light scattering occurs in air instead of in water and since the refractive index of air is less than that of water, more scattering will occur and the lesion is shown more clearly [Theuns et al., 1993].

Using measurements of photon path-length distribution of light inside sound and carious enamel with Monte Carlo simulations, Mujat et al [Mujat et al., 2003] found that the light paths inside both carious and sound enamel are considerably influenced by refractive index contrast. Clinically, the rapid dehydration of incipient lesions under airflow, resulting in a white spot appearance, is used to diagnose caries.

If quantification of fluorescence is influenced by the degree of hydration of the tissue, this would hamper an accurate assessment of the mineral loss as is currently done in clinical studies [Al-Khateeb et al., 1998; Tranaeus et al., 2001]. Data on the effects of
dehydration on enamel fluorescence were published by Angmar-Mansson and ten Bosch [Angmar-Mansson and ten Bosch, 2001] who performed a short-term in vitro study which was prompted by the initial qualitative observations presented by Al-Khateeb [Al-Khateeb et al., 1998]. Angmar-Mansson and ten Bosch found that dehydration increases the fluorescence loss in vitro by a factor of 0.1 to 0.15. Drying in a stream of air caused dehydration within seconds, reaching a maximum after about 15 minutes. Pretty et al [Pretty et al., 2004b] examined the effect of dehydration on demineralised lesions of varying severity and tried to determine an optimal drying method to obtain reliable quantitative light-induced fluorescence (QLF) readings. They found that compressed air-drying for 15 s produced reliable and predictable results with both distilled water and saliva-hydrated lesions.
6.3 Objectives and Working Hypothesis

1) To quantify the loss of fluorescence in QLF and the changes in the backscattered intensity of OCT in early enamel erosion when a water-moistened eroded surface is used as reference.

2) To compare the water-moistened eroded surface reference technique with a dentine bonding agent coated reference technique.

The Null Hypothesis tested were:

I) A water-moistened eroded surface cannot be used as a reference in the *in vitro* longitudinal monitoring of early enamel erosion with QLF.

II) A water-moistened eroded surface reference technique is not as sensitive as the coated surface reference technique in the monitoring of early enamel erosion with QLF.

III) A water-moistened eroded surface cannot be used as a reference in the *in vitro* longitudinal monitoring of early enamel erosion with OCT.

IV) A water-moistened eroded surface reference technique is not as sensitive as the coated surface reference technique in the monitoring of early enamel erosion with OCT.

V) A reference area is needed in the longitudinal monitoring of early enamel erosion with OCT *in vitro.*
6.4 Materials and Methods

6.4.1 Sample preparation and erosion cycle

Convenience sample of 12 extracted upper or lower human central incisors, caries free, with no visually obvious enamel fluorosis, were selected. The teeth were collected by the Kerala Dental School, Kerala, India and were stored in thymolised water. Each tooth was fully embedded in a separate cold cured methyl-methacrylate resin block with dimensions of 10 mm x 10 mm x 30 mm, except for a 5 mm x 5 mm window at the middle third of its labial surface. The incisors were mounted such that the level of the exposed labial surface was ± 1mm from the adjacent resin. This was to ensure optimum contact with the orange juice and the consistency of height variation to the depth of field of the QLF system. One half of the exposed labial surface was protected with a non-residue masking tape whilst the other half was being coated with a one-step self-etch adhesive, Xeno® V (DENTSPLY) according to the manufacturer’s instructions. This coated reference area will be referred to hereafter as the non-exposed (NE) surface, which is the surface that is used to account for any instrumental or systematic errors. Upon the completion of application of Xeno® V (DENTSPLY), the masking tape was removed from the other half of the labial surface so that it is left exposed to the erosive challenge.

The samples were dried for 20 seconds [Pretty et al., 2004b] with compressed air fixed 10 cm away from the resin block. A QLF image of the air-dried sample was first taken. Immediately following that and without removing the samples from the positioning jig, the exposed surface was moistened with a micro brush saturated with water. The sample was left for 10 seconds to allow permeation of water through the enamel surface and another QLF image was taken. This re-moistened reference area was an attempt to simulate sound enamel and will be referred to hereafter as the moistened-exposed (ME) area. This drying and moistening cycle was repeated for the capturing of OCT images.
For the erosive challenge, the teeth samples in the resin blocks were suspended with plastic rods, into a commercially available orange juice (ASDA Orange Juice from Concentrate) (pH 3.8±0.1). Two groups of six teeth samples were each suspended in 500 ml of orange juice gently agitated with a magnetic stirrer. The pH of the orange juice was monitored throughout the erosion cycle. After every 10 minutes for up to a total of 60 minutes, the teeth were removed from the orange juice and rinsed under a reservoir of running water for 1 minute to remove excess acid from their surface. Two sets of QLF and OCT images were then taken again following the drying and moistening protocol described above.

### 6.4.2 QLF

The same QLF set up, analysis program and method as described in Chapter 5 were used for the QLF measurements. The mean pixel value of the green channel was obtained for the defined ROI for the exposed, \( F_E(t) \), the moistened exposed, \( F_{ME}(t) \) and non-exposed areas, \( F_{NE}(t) \). All images were analysed blind to the erosive challenge intervals, \( t \) and to the type of reference method used.

The outcome measures defined for QLF for this study are

i) The percentage loss of fluorescence of the exposed area, with the non-exposed area as reference, \( \Delta F_1(t) \), was calculated as follows:

\[
\Delta F_1(t) = 100 \left( \frac{F_E(t)}{F_E(t_0)} - \frac{F_{NE}(t)}{F_{NE}(t_0)} \right)
\]

ii) The percentage loss of fluorescence of the exposed area, with the moistened exposed area as reference, \( \Delta F_2(t) \), was calculated as follows:

\[
\Delta F_2(t) = 100 \left( \frac{F_E(t)}{F_E(t_0)} - \frac{F_{ME}(t)}{F_{ME}(t_0)} \right)
\]

with \( t_0 \) being the baseline time point, and \( t \), the various erosive challenge time points.
6.4.3 OCT

The same OCT system, analysis program and method described in Chapter 5 were used to capture and analyse the OCT images.

The outcome measures defined for OCT for this study were:

i) $D_E$, the decay of backscattered light of the exposed area. It is the relationship of the attenuation of backscattered light between two optical depths and is represented by the function

$$D_E(t) = \frac{I_{\text{plateau}}(t)}{I_{\text{superficial}}(t)}$$

$I_{\text{superficial}}$ is the intensity of backscattered light immediately below the tooth-air interface and $I_{\text{plateau}}$ is the backscattered intensity where the A-scans had reached a plateau and is assumed not to be affected by the erosive challenge.

ii) $\Delta D_1(t)$, the percentage difference of decay between the exposed ($D_E$) and non-exposed ($D_{NE}$) areas, is represented by the function

$$\Delta D_1(t) = 100 \left[ \frac{D_E(t)}{D_E(t_0)} - \frac{D_{NE}(t)}{D_{NE}(t_0)} \right]$$

iii) $\Delta D_2(t)$, the percentage difference of decay between the exposed ($D_E$) and moistened-exposed ($D_{ME}$) areas, is represented by the function

$$\Delta D_2(t) = 100 \left[ \frac{D_E(t)}{D_E(t_0)} - \frac{D_{ME}(t)}{D_{ME}(t_0)} \right]$$

with $t_0$ being the baseline time point and $t$ the various erosive challenge time points.

Results in Chapter 5 demonstrated that the backscattered light intensity at the enamel-air interface and 10 µm below the interface was confounded by specular reflection and did not show erosion-interval related changes. Therefore the chosen superficial optical
depths for this study were 20 µm, 30 µm, 40 µm and 50 µm below the tooth-air interface and the optical depth where the backscattered light plateau was situated at 150 µm.

6.4.4 Statistical analysis

The STATA$$^{\text{TM}}$$ 10.1 statistical program was used. Multiple linear regression analyses were performed, taking into account the clustering of samples using robust variance estimates, to ascertain whether time-related changes were detected with the different reference methods for QLF and OCT.

When a significant time-related change was found for an outcome measure, paired t-test was performed to identify the detection sensitivity, which is defined as the shortest subsequent time intervals when a significant difference was detected.

6.5 Results

6.5.1 QLF

6.5.1.1 Reference - Non-exposed surface, $\Delta F_1$

Multiple regression analysis of $\Delta F_1$ with erosive challenge intervals showed significant ($P < 0.05$) time-related loss of fluorescence. A linear curve could be fitted to it with an $R^2$ value of 0.594.

Paired t-tests were used to analyse the differences of the loss of fluorescence between each consecutive interval of erosion. Significant differences ($P<0.05$) were first detected between baseline and 10 minutes erosion and thereafter every consecutive 20 minutes of erosive challenge (10 – 30 minutes, 30 – 50 minutes) as well as between the last two 10-minute time points of erosion (Table 6.1).

Figure 6.1 shows the mean percentage loss of fluorescence calculated for the two different reference methods throughout the erosive challenge. $\Delta F_1(t)$ showed that
there was 1.33 +/- 0.50 (mean +/- SE) of mean percentage loss of fluorescence during
the first 10 minutes of erosion and cumulatively 8.90 +/- 0.76 percentage fluorescence
loss at the end of 60 minutes of erosion.

6.5.1.2 Reference - Moistened-exposed surface, $\Delta F_2$

Multiple regression analysis of $\Delta F_2$ with erosive challenge intervals also showed
significant (P < 0.05) time-related loss of fluorescence, although with a lower $R^2$ value
(0.333) compared to that of $\Delta F_1$.

Paired t-tests were used to analyse the differences of the loss of fluorescence between
each consecutive interval of erosion. Significant differences (P<0.05) were first
detected only after 30 minutes of erosive challenge and subsequently at 50 minutes of
erosive challenge. No significant difference was detected between the last two 10-
minute time points of erosive challenge (Table 6.1). It was found that the mean
percentage loss of fluorescence calculated with moistened surface as the reference
was only 0.87 +/- 1.11 (mean +/- SE) during the first 10 minutes of erosion and
cumulatively 5.72 +/- 0.73 at the end of 60 minutes of erosion (Figure 6.1).

Figure 6.1 illustrates the similar trend of increase of loss of fluorescence of both $\Delta F_1$
and $\Delta F_2$ as erosion progressed. Compared to $\Delta F_1$, $\Delta F_2$ demonstrated smaller mean
percentage losses throughout the whole duration with mean percentage loss of
fluorescence reaching 3.00 +/- 0.93 % after 30 minutes of erosion as compared to
$\Delta F_1$'s 4.97 +/- 0.58 %. Loss of fluorescence detected with $\Delta F_2$ started to plateau after
50 minutes of erosive challenge, at approximately 5.7 % while $\Delta F_1$ did not show signs
of plateau. Larger standard deviations were also observed with $\Delta F_2$. 
6.5.2 OCT

6.5.2.1 No reference area used, $D_E$

Multiple linear regression analysis showed that there were significant erosion-interval related decrease of $D_E$ for the four depth combinations of 150 µm/20 µm, 150 µm/30 µm, 150 µm/40 µm and 150 µm/50 µm ($P < 0.05$) (Table 6.2). $D_E$ (150 µm/20 µm) demonstrated the highest $R^2$ value of 0.300 and was followed by $D_E$ (150 µm/30 µm) with a value of 0.282. The graph in Figure 6.2 shows the mean attenuation decay, $D_E$, with time of the four optical depth combinations. It was observed that for combinations 150 µm/20 µm and 150 µm/30 µm, the decrease of the mean $D_E$ was greater during the first 50 minutes of erosion but started to plateau thereafter. As for combinations 150 µm/40 µm and 150 µm/50 µm, the decreases were smaller during the first 20 minutes of erosion but became more pronounced after that, except for the final 10 minutes when it started to plateau.

Paired t-tests were performed to determine the detection threshold demonstrated by the four optical combinations with $D_E$ and the results are presented in Table 6.3. The $D_E$ for depth combinations of 150 µm/20 µm and 150 µm/30 µm showed significant difference in decay at three time points while those of 150 µm/40 µm and 150 µm/50 µm showed significant difference in decay at two time points.

$D_E$ for depth combination 150 µm/20 µm was the first to detect significant decrease of decay at 10 minutes but did not detect a subsequent significant decrease until at 40 minutes and 50 minutes of erosive challenge. $D_E$ for combination 150 µm/30 µm on the other hand demonstrated significant decrease of decay at 20, 40 and 50 minutes of erosive challenge. $D_E$ for combinations 150 µm/40 µm and 150 µm/50 µm demonstrated identical detection threshold first at 30 minutes than at 50 minutes of erosive challenge.
6.5.2.2 Reference - Non-exposed surface, $\Delta D_1$

Multiple linear regression analysis showed that there were significant erosion-interval related changes in $\Delta D_1$ for all the four depth combinations of 150 µm/20 µm, 150 µm/30 µm, 150 µm/40 µm and 150 µm/50 µm (P <0.05) (Table 6.2). $\Delta D_1$ (150 µm/50 µm) demonstrated the highest $R^2$ value of 0.319 followed by $\Delta D_1$ (150 µm/30 µm) with a value of 0.312.

The graph in Figure 6.3 illustrates the progression of $\Delta D_1$ throughout the erosive challenge for all the depth combinations. As the erosive challenge progressed, a decrease in difference of decay between the exposed and non-exposed areas was observed for all depth combination except for combination 150 µm/20 µm where there was a sudden increase at 50 minutes. $\Delta D_1$ for combinations 150 µm/40 µm and 150 µm/50 µm were greatest between 20 and 30 minutes and the trend of decrease continued up till the end of the erosive challenge with the depth combination 150 µm/40 µm increasing slightly more than 150 µm/50 µm. $\Delta D_1$ for depth combinations 150 µm/20 µm and 150 µm/30 µm on the other hand were greatest between baseline and 10 minutes (24.17% and 41.07% respectively). However, although $\Delta D_1$ for both of these combinations demonstrated a net decrease at the end of 60 minutes, only $\Delta D_1$ for combination 150 µm/30 µm demonstrated a steady decrease throughout erosive challenge, while that of combination 150 µm/20 µm showed an erratic trend of decreased change after 40 minutes of erosive challenge.

Paired t-tests were performed to evaluate the detection sensitivity of the four depths combinations used $\Delta D_1$ and the results are presented in Table 6.4. The $\Delta D_1$ for depth combinations of 150 µm/30 µm and 150 µm/40 µm both showed significant difference in decay at three time points while those of 150 µm/20 µm and 150 µm/50 showed significant difference in decay at two time points. $\Delta D_1$ of 150 µm/30 µm demonstrated
early detection sensitivity at 10 minutes but subsequently only at 50 minutes and 60 minutes of erosive challenge. \( \Delta D_1 \) for 150 \( \mu m/40 \) \( \mu m \) demonstrated detection sensitivity at every 20 minutes interval and 150 \( \mu m/50 \) \( \mu m \) at two 30 minutes interval.

6.5.2.3 Reference - Moistened-exposed surface, \( \Delta D_2 \)

Multiple linear regression analysis showed that there were significant erosion-interval related changes in \( \Delta D_2 \) for the depth combinations of 150 \( \mu m/30 \) \( \mu m \), 150 \( \mu m/40 \) \( \mu m \) and 150 \( \mu m/50 \) \( \mu m \) (P <0.05) (Table 6.2) but the R\(^2\) value were much smaller compared to those observed for \( D_E \) and \( \Delta D_1 \). Similar to \( \Delta D_1 \), \( \Delta D_2 \) for combination 150 \( \mu m/50 \) \( \mu m \) demonstrated the highest R\(^2\) value of 0.069 followed by \( \Delta D_2 \) (150 \( \mu m/30 \) \( \mu m \)) with a value of 0.062.

The graph in Figure 6.4 illustrates the progression of \( \Delta D_2 \) throughout the erosive challenge for all the depth combinations. For the first 10 minutes of erosive challenge, decreases of \( \Delta D_2 \) were observed for all depth combinations with 150 \( \mu m/20 \) \( \mu m \) demonstrating the largest decrease of 39.40 ± 12.30 %. From 10 minutes up till 40 minutes of erosive challenge, though there were net decreases in \( \Delta D_2 \) for all depth combinations, only the \( \Delta D_2 \) for 150 \( \mu m/30 \) \( \mu m \) demonstrated a distinct trend of decrease. During the last 20 minutes of erosive challenge, all depth combinations demonstrated a increase in \( \Delta D_2 \).

Paired t-tests were performed to evaluate the detection sensitivity of the four depths combination using \( \Delta D_2 \) and the results are presented in Table 6.5. \( \Delta D_2 \) for all depth combinations showed significant decrease in decay at only one time point throughout the erosive challenge period. \( \Delta D_2 \) for the combination of 150 \( \mu m/20 \) \( \mu m \), 150 \( \mu m/30 \) \( \mu m \) and 150 \( \mu m/40 \) \( \mu m \) detected significant decrease after 10 minutes erosive challenge while combination 150 \( \mu m/50 \) \( \mu m \) detected this after 30 minutes.
6.6 Discussion

6.6.1 QLF
Less loss of fluorescence was detected with the moistened-exposed surface reference technique, $\Delta F_2$, compared to that of the non-exposed surface technique, $\Delta F_1$. This was probably related to the observation that at baseline, the moistened-exposed surfaces were consistently more fluorescent than the Xeno-covered surface. $\Delta F_2$ also manifested larger standard deviations. These large standard deviations demonstrated could be due to a difference in the diffusion rate of water into the surface porosity in different samples and 10 seconds of waiting time prior to taking a QLF image may not be sufficient for a uniform permeation of water into the porosity of each sample.

However, $\Delta F_2$ showed similar trend and order of magnitude of fluorescence loss as $\Delta F_1$. This indicates that both reference methods are suitable to be used for the longitudinal monitoring of demineralisation up to 60 minutes of in vitro demineralisation but $\Delta F_1$ is more sensitive in discriminating smaller changes, as shown in Table 6.1.

6.6.2 OCT
The increase in porosity during initial demineralization causes an increase in the backscattered intensity within the top layers immediately beneath the enamel-air interface. Consequently, the backscattered intensity of the deeper layers reduces. This phenomenon is illustrated in the B-scans (Figure 6.5) and the corresponding A-scans (Figure 6.6) of a specimen. Hence, as demineralization progressed, the decay of backscattered light, $D_E$ decreased.

6.6.2.1 Comparison of the three OCT outcome measures
Although multiple linear regression analysis of all three outcome measures with erosion interval showed significant time related changes, the $R^2$ value $\Delta D_2$ is much lower in magnitude than that of $\Delta D_1$ and $D_E$ for all the four depth combinations (Table 6.2).
This implies that the linear relationship of $\Delta D_2$ with 60 minutes of erosive challenge is weak, and $D_E$ and $\Delta D_1$ are more suited to detect demineralisation changes of 60 minutes in vitro erosive challenge with orange juice.

Paired t-tests results of $\Delta D_2$ for combination of 150 µm/20 µm, 150 µm/30 µm and 150 µm/40 µm were only sensitive during the first 10 minutes of demineralisation and are not useful for the quantification of demineralisation beyond that time point. This phenomenon could be due to the fact that during the first stages of demineralisation when only the prism sheath regions are affected, the replacement of the minerals of these sheaths with water (refractive index 1.3) maintained the refractive index of a moistened-eroded enamel surface close to the refractive index of sound enamel. Hence a moistened-eroded enamel surface was able to serve as a reference to the dried-eroded surface for the first 10 minutes. As demineralisation progress to the prism cores and more water replaced the lost minerals, the discrepancy between the refractive index of sound enamel and moistened-eroded enamel becomes more pronounced and the moistened-exposed surface can no longer simulate the refractive index of sound enamel. Although there is no strong relationship between $\Delta D_2$ and 60 minutes acid challenge, $\Delta D_2$ for the first 10 minutes of erosion (for all depth combinations) showed more changes than $\Delta D_1$. For example, during the 10 minutes of erosive challenge, $\Delta D_2$ (150 µm/20 µm) was -33.42 ± 12.72 while $\Delta D_1$ (150 µm/20 µm) was only -17.68 ± 11.14 and $\Delta D_2$ (150 µm/40 µm) was -17.00 ± 7.13 while $\Delta D_1$ (150 µm/40 µm) was 0.55 ± 11.43. It can be postulated that $\Delta D_2$ might be more sensitive than $\Delta D_1$ in the first 10 minutes of erosion and if so could provide valuable information regarding the very early stages of the erosion process. More work needs be done to explore this possibility.
6.6.2.2 Comparison between $D_E$ and $\Delta D_1$

Between $D_E$ and $\Delta D_1$, $\Delta D_1$ is a more sensitive outcome measure than $D_E$ in the longitudinal monitoring of demineralisation as it demonstrated a higher $R^2$ value than $D_E$ (Table 6.2). This was to be expected as the decay of backscattered intensity from the non-exposed area, $D_{NE}$, was used in the calculations to compensate for any fluctuations in the intensity of the incident light throughout the study period. The non-exposed area is not affected by erosion and the backscattered intensity should remain relatively constant throughout the period of the study. Any observed variation in the backscattered intensity would be due to variability of the intensity of the incident light. Figure 6.7 shows the decay of backscattered light of the non-exposed area, $D_{NE}$, of the various depth combinations. The decay of the backscattered light for all the four depth combinations at the non-exposed area $D_{NE}$ was relatively constant from baseline through to 50 minutes of erosive challenge. However, an increase in $D_{NE}$ of all four depth combinations was observed during the last 10 minutes of the erosion challenge. This suggests that there could have been fluctuations to the intensity of the incident light during these last 10 minutes but the outcome measure $\Delta D_1$, had compensated for these fluctuations.

If the output of the incident light was monitored and maintained at a constant intensity throughout the study period, $D_E$ would be an acceptable outcome measure. With $D_E$ as an acceptable outcome measure, studies could be conducted without needing a reference area. Longitudinal monitoring of changes in the degree of demineralisation is possible by comparing $D_E$ of a time point with that of the baseline. Eliminating the necessity for a reference area has the advantages of reducing error such as from possible deterioration of the coating [Azzopardi et al., 2004] and uneven thickness of coating. This would make the study design simpler, reduce costs and shorten the study time.
The validity of $D_E$ also makes OCT a potential clinical diagnostic tool to distinguish erosive wear from other forms of tooth wear \textit{in vivo} (or confirming the occurrence of the erosion process in a lesion). Eisenburger et al [Eisenburger et al., 2004] has suggested from an SEM study that the deeper parts of the softened enamel, where demineralisation appears to be mostly confined to the prism boundaries, might have similar physical resistance as sound enamel to ultrasonication. Therefore, residual softened enamel could still be present on tooth surfaces when the outermost, more severely demineralised top layers had been removed by mechanical forces such as abrasion. With the presence of this residual softened enamel, a reduced decay of backscattered intensity ($D_E$) could be detected when compared to the adjacent areas of the tooth which have no apparent presentation of wear. Lesions resulting from pure attrition or abrasion will not exhibit a reduced $D_E$.

6.6.2.3 Comparing the various depth combinations

Multiple linear regression analysis showed that the $R^2$ value at the depth combination of 150 µm/30 µm for all three outcome measures were consistently one of the highest. This indicates that the OCT set-up used in this study is most sensitive to the demineralisation activity that occurs in this level. Backscattered intensity from lesion levels more superficial to it were most likely confounded by specular reflection. This concurs with the findings in Chapter 5.

The optical depth of 30 µm translates to 18.75 µm of physical depth measured from the tooth-air interface. This means that demineralisation occurs all the way into this depth.

In 2000, Eisenburger et al [Eisenburger et al., 2000] using ultrasonification and contact profilometry, found that subsurface softening occurs only 2 – 4 µm into enamel. However, subsequent to that in 2004, with a freeze-drying technique, they [Eisenburger et al., 2004] found that the previous findings underestimated the depth of subsurface softening. They examined replicas of subsurface pores, created by resin impregnation
with SEM and found that acid damage of 5 – 20 minutes extended to a depth of more than 9 – 12 µm below the surface and even this depth was an underestimate, as the most demineralised part of the softened layer is not detectable within the resin. The findings of Eisenburger et al are echoed by this study.

6.6.3 Comparing QLF and OCT

For QLF, the current method of moistening the eroded surface with water using a water-saturated micro brush and 10 seconds of permeation time sufficiently approximated the refractive index of eroded enamel to that of sound enamel throughout the whole study interval of 60 minutes erosive challenge. This is demonstrated by the relatively high $R^2$ value of both the non-exposed and moistened-exposed surface technique.

However this was not the case for OCT. This is shown by the low $R^2$ value of $\Delta D_2$ and the detection of significant difference in decay only during the first 10 minutes of erosive challenge. This phenomenon could be due to two reasons. Firstly, the backscattered intensity from the superficial 10 - 20 µm of eroded enamel were not taken into consideration for OCT measurements due to the confounding effect of specular reflection. Secondly, the 10 seconds of permeation time might have only been sufficient to rehydrate the superficial layers of eroded enamel. A longer permeation time may be necessary to improve the sensitivity of this technique.

The QLF set-up ensured that the fluorescence signal was not affected by specular reflection and the effect of the reduction of scattering due to the imbibitions of water into the porous superficial layers was detected.
6.7 Conclusion

I) A water-moistened eroded surface can be used as a reference in the *in vitro* longitudinal monitoring of 60 minutes erosive challenge using QLF.

II) A water-moistened eroded surface reference technique is not as sensitive as the coated surface reference technique in the *in vitro* monitoring of early enamel erosion with QLF.

III) A water-moistened eroded surface cannot be used as a reference in the *in vitro* longitudinal monitoring of 60 minutes erosive challenge using OCT.

IV) A reference area is not needed in the longitudinal monitoring of early enamel erosion with OCT *in vitro*.
6.8 References


6.9 Tables

Table 6.1
Detection Sensitivity of outcome measure of QLF

<table>
<thead>
<tr>
<th>Outcome Measure</th>
<th>10 minutes</th>
<th>20 minutes</th>
<th>30 minutes</th>
<th>40 minutes</th>
<th>50 minutes</th>
<th>60 minutes</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\Delta F_1$</td>
<td>P=0.023</td>
<td>P=0.000</td>
<td></td>
<td>P=0.013</td>
<td>P=0.001</td>
<td></td>
</tr>
<tr>
<td>$\Delta F_2$</td>
<td>P=0.009</td>
<td></td>
<td>P=0.022</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Results of paired t-tests, used to analyse the differences of the loss of fluorescence between each consecutive interval of erosion.

The percentage loss of fluorescence of the exposed area, with the non-exposed area as reference, $\Delta F_1$, showed significant differences (P<0.05) between baseline and 10 minutes erosion and thereafter every consecutive 20 minutes of erosive challenge (10 – 30 minutes, 30 – 50 minutes) as well as between the last two 10-minute time points of erosion.

The percentage loss of fluorescence of the exposed area, with the moistened exposed area as reference, $\Delta F_2$, first showed significant differences (P<0.05) only after 30 minutes of erosive challenge and subsequently at 50 minutes of erosive challenge. No significant difference was detected between the last 10 minutes of erosive challenge.
### Table 6.2

**Multiple Linear Regression Analysis of the outcome measures of OCT with Erosive Challenge Interval**

<table>
<thead>
<tr>
<th>Optical depths combination</th>
<th>$D_E$</th>
<th>$\Delta D_1$</th>
<th>$\Delta D_2$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$R^2$</td>
<td>P value</td>
<td>$R^2$</td>
</tr>
<tr>
<td>150 µm/ 20 µm</td>
<td>0.300</td>
<td>&lt; 0.000</td>
<td>0.172</td>
</tr>
<tr>
<td>150 µm/ 30 µm</td>
<td>0.282</td>
<td>&lt; 0.000</td>
<td>0.312</td>
</tr>
<tr>
<td>150 µm/ 40 µm</td>
<td>0.175</td>
<td>&lt; 0.000</td>
<td>0.305</td>
</tr>
<tr>
<td>150 µm/ 50 µm</td>
<td>0.117</td>
<td>0.002</td>
<td>0.319</td>
</tr>
</tbody>
</table>

Significant erosion-interval related decrease (P <0.05) of $D_E$, $\Delta D_1$ and $\Delta D_2$ were observed for the four depth combinations of 150 µm/20 µm, 150 µm/30 µm, 150 µm/40 µm and 150 µm/50 µm except for $\Delta D_2$ (150 µm/20 µm).

$D_E$ (150 µm/20 µm) demonstrated the highest $R^2$ value of 0.300 while $\Delta D_1$ (150 µm/30 µm) and $\Delta D_1$ (150 µm/50 µm) demonstrated similar $R^2$ value.
Table 6.3
Detection Sensitivity of OCT using the outcome measure, $D_E$.

<table>
<thead>
<tr>
<th>Optical depths combination</th>
<th>10 minutes</th>
<th>20 minutes</th>
<th>30 minutes</th>
<th>40 minutes</th>
<th>50 minutes</th>
<th>60 minutes</th>
</tr>
</thead>
<tbody>
<tr>
<td>150 μm/20 μm</td>
<td>P=0.020</td>
<td></td>
<td>P=0.026</td>
<td>P=0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>150 μm/30 μm</td>
<td>P=0.012</td>
<td>P=0.005</td>
<td>P=0.008</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>150 μm/40 μm</td>
<td>P=0.005</td>
<td></td>
<td></td>
<td>P=0.007</td>
<td></td>
<td></td>
</tr>
<tr>
<td>150 μm/50 μm</td>
<td>P=0.038</td>
<td></td>
<td></td>
<td></td>
<td>P=0.002</td>
<td></td>
</tr>
</tbody>
</table>

Results of paired t-tests, used to analyse $D_E$, the decay of backscattered light of the exposed area between each consecutive interval of erosion.

The $D_E$ for depth combinations of 150 μm/20 μm and 150 μm/30 μm showed significant difference in decay at three time points while those of 150 μm/40 μm and 150 μm/50 showed significant difference in decay at two time points.
Table 6.4

Detection Sensitivity of OCT using the outcome measure, $\Delta D_1$.

<table>
<thead>
<tr>
<th>Optical depths combination</th>
<th>10 minutes</th>
<th>20 minutes</th>
<th>30 minutes</th>
<th>40 minutes</th>
<th>50 minutes</th>
<th>60 minutes</th>
</tr>
</thead>
<tbody>
<tr>
<td>150µm/20µm</td>
<td>P=0.023</td>
<td></td>
<td></td>
<td></td>
<td>P=0.024</td>
<td></td>
</tr>
<tr>
<td>150µm/30µm</td>
<td>P=0.005</td>
<td></td>
<td>P=0.001</td>
<td></td>
<td></td>
<td>P=0.049</td>
</tr>
<tr>
<td>150µm/40µm</td>
<td>P=0.026</td>
<td></td>
<td>P=0.034</td>
<td></td>
<td></td>
<td>P=0.028</td>
</tr>
<tr>
<td>150µm/50µm</td>
<td></td>
<td>P=0.005</td>
<td></td>
<td></td>
<td></td>
<td>P=0.029</td>
</tr>
</tbody>
</table>

Results of paired t-tests, used to analyse $\Delta D_1$, the decay of backscattered light of the exposed area between each consecutive interval of erosion.

The $\Delta D_1$ for depth combinations of 150 µm/30 µm and 150 µm/40 µm both showed significant difference in decay at three time points while those of 150 µm/20 µm and 150 µm/50 showed significant difference in decay at two time points.
Table 6.5

Detection Sensitivity of OCT using the outcome measure, $\Delta D_2$

<table>
<thead>
<tr>
<th>Optical depths combination</th>
<th>10 minutes</th>
<th>20 minutes</th>
<th>30 minutes</th>
<th>40 minutes</th>
<th>50 minutes</th>
<th>60 minutes</th>
</tr>
</thead>
<tbody>
<tr>
<td>150 μm/20 μm</td>
<td>P=0.024</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>150 μm/30 μm</td>
<td>P=0.028</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>150 μm/40 μm</td>
<td>P=0.036</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>150 μm/50 μm</td>
<td>P=0.002</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Results of paired t-tests, used to analyse $\Delta D_2$, the decay of backscattered light of the exposed area between each consecutive interval of erosion.

$\Delta D_2$ for all depth combinations showed significant decrease in decay at only one time point throughout the erosive challenge period.
6.10 Figures

Figure 6.1
Mean percentage loss of fluorescence of the two different reference methods (A - Non-exposed reference method, B - Moistened-exposed reference method) during the erosive intervals. Both methods showed similar percentage loss of fluorescence at the end of the 60 minutes erosion challenge ($\Delta F_1$, 8.90 ± 0.76 and $\Delta F_2$, 8.13 ± 2.50) but the moistened-exposed reference technique demonstrated higher standard error.
Figure 6.2

Figure shows the decay of backscattered light of the exposed area, $D_E$, of the 4 depth combinations (150 µm/20 µm, 150 µm/30 µm, 150 µm/40 µm and 150 µm/50 µm) which demonstrated significant erosion-interval related changes. $D_E$ of all these four depth combinations decreased as erosion progressed with $D_E$ (150 µm/30 µm) demonstrating the most decrease. However, the difference in decrease among the four depth combinations were not significantly different.
Figure 6.3

Figure shows the decay of backscattered light of $\Delta D_1$, the percentage difference of decay between the exposed ($D_E$) and non-exposed ($D_{NE}$) areas of the 4 depth combinations (150 µm/20 µm, 150 µm/30 µm, 150 µm/40 µm and 150 µm/50 µm) that demonstrated significant erosion-interval related changes. All four depth combinations demonstrated a decrease of $\Delta D_1$. At the end of 60 minutes erosive challenge, $\Delta D_1$ (150 µm/30 µm) showed the largest decrease (44.9 ± 7.04 %), followed by $\Delta D_1$ (150 µm/40 µm) and $\Delta D_1$ (150 µm/50 µm) (36.2 ± 6.1% and 32.4 ± 5.3 % respectively).
Figure 6.4

Figure shows the decay of backscattered light of $\Delta D_2$, the percentage difference of decay between the exposed ($D_E$) and moistened-exposed ($D_{ME}$) areas of 4 depth combinations (150 µm/20 µm, 150 µm/30 µm, 150 µm/40 µm and 150 µm/50 µm). For the first 10 minutes of erosive challenge, decreases of $\Delta D_2$ were observed for all depth combinations with 150 µm/20 µm demonstrating the largest decrease of 39.40 ± 12.30%. However from 10 minutes up till 40 minutes, only $\Delta D_2$ (150 µm/30 µm) demonstrated a distinct trend of decrease. During the last 20 minutes of erosive challenge, all depth combinations demonstrated a increase in $\Delta D_2$. 
Figure 6.5
Figure shows OCT B-scans of a sample taken after 20 minutes of air drying at baseline, 30 minutes erosion and at the end of the 60 minutes erosion. The backscattered intensity of the non-exposed side (NE) remain the same while there is increased backscattering intensity at the surface of the exposed (E) side as erosion progresses.
Figure 6.6

The figure shows mean A-scans of the exposed side of a sample at baseline, 20, 30 and 50 minutes of erosive challenge. The optical depths of $I_{\text{superficial}}$ chosen for the study were 0, 10, 20, 30, 40 and 50µm from the tooth-air interface. The observed optical depth for $I_{\text{plateau}}$ where the backscattered intensity reached a plateau was 150µm.
Figure 6.7

Figure shows the decay of backscattered light of the non-exposed area, $D_{NE}$, of the various depth combinations. The decay of the backscattered light for all the four depth combinations at the non-exposed area $D_{NE}$ was relatively constant from baseline through to 50 minutes of erosive challenge. However, an increase in $D_{NE}$ of all four depth combinations was observed during the last 10 minutes of the erosion challenge. This suggests that there could have been fluctuations to the intensity of the incident light during these last 10 minutes but the outcome measure $\Delta D_1$, had compensated for these fluctuations.
CHAPTER 7

MEASURING EARLY ENAMEL EROSION WITH OCT & QLF \textit{in situ}.

Part I: PRIMARY OUTCOMES – VALIDATION
7.1 Abstract

Increased porosity in early eroded enamel before surface loss (60 minutes of \textit{in vitro} erosive challenge of orange juice), as demonstrated in Chapter 5, can be quantified using Optical Coherence Tomography (OCT) and Qualitative Light-Induced Fluorescence (QLF) \textit{in vitro}. However, clinically, erosion rate is likely to be slower than \textit{in vitro} due to the protective effect of saliva and pellicle.

**Objectives:** The aim of this study was to investigate the sensitivity of OCT and QLF in detecting early erosion \textit{in situ} and to validate them against surface microhardness.

**Methods:** 20 volunteers wore a mandibular appliance with four embedded human enamel samples, three with natural surfaces (Ns) and one with polished surface (Ps), across a study period of 35 days. Each day the participants wore the appliances from 09.00 to 16.00 and swished a total of 250 ml orange juice around their mouth for 10 minutes at three separate intervals. Three dimensional OCT images, QLF images and micro indents with a Knoop micro indenter were made at baseline and at the end of each day.

**Results:** Percentage of surface microhardness change ($\Delta SMC$), decay of backscattered intensity of OCT ($\Delta D$) and percentage loss of fluorescence of the exposed area ($\Delta F$) were analysed. Multiple regression analysis of erosion interval with $\Delta SMC$, $\Delta D$ and $\Delta F$ showed that surface microhardness, OCT and QLF detected significant erosion-interval related changes on both natural and polished surfaces. There was also a significant but weak correlation between $\Delta SMC$ and $\Delta D$ and between $\Delta SMC$ and $\Delta F$, for both natural surface and polished surface (P<0.001), with Pearson correlation coefficients of 0.351 and 0.572 respectively.
7.2 Introduction

Like dental caries, the aetiology of dental erosion is multifactorial and the rate at which the process occurs is dependent on the interplay between biological, chemical and behavioural factors [Lussi, 2006].

7.2.1 Saliva

The biological factor that is well recognised as the most important in determining the rate of erosion is saliva. Studies have shown that erosion is strongly associated with low salivary flow and low buffering capacity [Jarvinen et al., 1991; Meurman et al., 1994; Rytomaa et al., 1998]. Its protective effect is two pronged. The first is its direct effect on the erosive agent and secondly is its role in the production and maturation of the acquired pellicle on the tooth surface.

Saliva reduces the rate of erosion by clearing away the acid, buffering the causative acid and by promoting remineralisation of any demineralisation that might have occurred due to previous erosive attack or is currently occurring.

The inorganic buffer systems in saliva consists of inorganic ions such as carbonic acid (H$_2$CO$_3$)/hydrogen carbonate (HCO$_3^-$), di-hydrogenphosphate (H$_2$PO$_4^-$)/hydrogen phosphate (HPO$_4^{2-}$), calcium (Ca$^{2+}$) and fluoride (F') [Dodds et al., 2005; Larsen and Pearce, 2003]. Stimulated saliva, when compared to resting saliva, has a much higher composition of these ions. The concentration of the principal buffer, hydrogen carbonate (HCO$_3^-$), has been shown to increase from 5mmol/l in resting saliva up to 60mmol/l in stimulated whole saliva [Dawes and Kubieniec, 2004].

The main production of stimulated saliva occurs during mastication. However, the production starts well before that with anticipatory salivary flow, as a response to the extra-oral stimuli such as odour or sight of sour foodstuff [Christensen and Navazesh, 1984; Engelen et al., 2003; Lee and Linden, 1992]. The taste of sour foodstuff also has a strong influence on the anticipatory salivary flow. Three droplets of 4% citric acid
applied to the tongue every 30s for 5 minutes caused the mean salivary flow rate to increase from 0.38ml/min to 1.87ml/min [Ferguson, 1975].

A high stimulated salivary flow rate not only increases the buffering capacity but also quickly clears away the offensive erosive agent before demineralisation progresses. The time required for saliva to neutralise and clear the acid from the tooth surface has been measured in vivo with pH electrodes and has shown to range between 2 and 5 minutes [Millward et al., 1997]. Bartlett et al [Bartlett et al., 2003] also reported similar ranges of 3 and 7 minutes but also reported wide individual variations.

The consistency of saliva has also been shown to provide different levels of protection against erosion [Dawes, 1987; Young and Khan, 2002]. Sites mainly bathed with mucous saliva (such as the labial surfaces of maxillary incisors) are more likely to show erosion when compared to sites bathed in serous saliva (lingual surfaces of mandibular teeth) [Young and Khan, 2002].

7.2.2 Pellicle

The acquired salivary pellicle is a biofilm, free of bacteria, covering oral soft and hard tissues. It abounds with protein, glycoproteins, enzymes and mucins [Lendenmann et al., 2000]. The formation of this organic layer is shown to be a two-step process [Skjorland et al., 1995]. Initially, adsorption of discrete proteins occurs by electrostatic interactions with the hydrophobic regions of the tooth, leaving hydrophilic parts of the protein molecules exposed at the surface. Following this initial process, protein aggregates may adsorb to uncovered sites on the tooth surface and also interact with the initially formed hydrophobic protein layer.

Two aspects of the pellicle have been extensively researched. The first is its role in microbial adherence and plaque formation and the second one being the role it plays in the protection of enamel against acid-induced demineralization.
Pellicle, be it formed \textit{in vitro} or \textit{in vivo}, has been shown in many studies to be able to protect enamel to some extent from demineralization when compared to specimens which were not covered with it. In an electron microscopic study, Meurman and Frank \cite{Meurman1991} demonstrated that the presence of pellicle, formed \textit{in vitro}, reduced erosion of enamel by an acidic Cola drink. Following that, similar results were obtained in studies of challenges with acetic acid \cite{Kautsky1993}, orange juice \cite{Amaechi1999} and 1\% citric acid \cite{Hannig2001}. The mechanism of this protection has been thought largely to be the pellicle acting as a diffusion barrier or a perm-selective membrane for acid diffusion. It prevents the direct contact between the acids and the tooth surface \cite{Hannig1999} and modifies the transport of calcium and phosphate ions into and out of the enamel surface \cite{Slomiany1986, Zahradnik1976}. It is also thought that the pellicle might serve as a reservoir of remineralising electrolytes \cite{Hannig2005}. Salivary enzymes like carbonic anhydrase VI has also been shown to play a protective role against dental erosion, by accelerating the neutralization of hydrogen ions on the tooth surface \cite{Hannig2005}. Nieuw Amerongen et al \cite{NieuwAmerongen1987} have also shown that mucins in the pellicle are able to enhance the protection of the enamel surface against demineralisation \cite{NieuwAmerongen1987}.

While the protective effect of the pellicle against demineralisation is non-controversial, the degree of the protection it provides had been found to be widely varied in different studies. The efficacy of the protection has been assessed either by the percentage of demineralisation that had occurred or by the extent of the demineralisation into enamel/dentine. It is dependent strongly on the thickness of the pellicle layer \cite{Amaechi1999}, the formation site and whether the pellicle was formed \textit{in vitro} or \textit{in vivo}.

Skjorland et al. \cite{Skjorland1995} reported that the final thickness of pellicle was attained within 30 min of oral exposure and stayed at that level for the 10 hrs of the experimental period. Similarly, Lamkin et al \cite{Lamkin1996} found that pellicle...
formation is a fairly rapid process, reaching a maximum after approximately 30 to 60 min. Hannig and Balz in a later study [Hannig and Balz, 1999] however, found both buccally and palatally formed pellicle increased in thickness approximately ten fold from 2 hours to 24 hours. They had reported that the age of the acquired pellicle is directly correlated with the thickness of the pellicle.

The above studies gave contradictory results in terms of the thickness and the optimum maturation age of pellicle that is effective against demineralisation and the time needed for pellicle to attain its full maturation. This is probably due to the many different experimental parameters used in the different studies, such as the type of acid used, the acid concentration, exposure time to acid and whether the acid challenge was induced intra or extra-orally. However, it is established that pellicles of different maturations levels do not provide complete protection against erosion.

It is also unanimously reported that pellicle formed in vivo is more effective in reducing the rate of demineralisation than those formed in vitro. Kautsky and Featherstone [Kautsky and Featherstone, 1993] found that an vivo 18 hours-old pellicle significantly reduced the dissolution of apatite discs while similarly aged in vitro pellicle did not. They continued the period of in vitro incubation and found that a 7 day old incubation in whole parotid saliva did reduce the dissolution of the apatite discs.

Hannig and Balz [Hannig and Balz, 1999] and Amaechi et al [Amaechi et al., 1999] both found a site difference in the thickness between buccally and palatally formed pellicle, with the palatally formed pellicle being much thinner. Hannig and Balz postulated that locally available salivary biopolymers and shearing forces affect the ultrastructural pattern and extent of pellicle formation. Nevertheless, this site-difference in thickness did not necessarily translate to better protection against acid erosion. Hannig and Balz in 2001 [Hannig and Balz, 2001] studied 24-hour pellicle formed near the opening of the parotid duct as well as the submandibular and sublingual ducts and their protective ability against erosion. They concluded that site-dependent differences
of in vivo formed pellicles have minor effects in terms of their protective ability against erosive changes. However, Amaechi et al [Amaechi et al., 1999], with 1-hour old pellicle found that in the lower arch, the level of erosion was significantly greater in the labial or buccal region compared with the lingual region. In the upper arch, the opposite was found where the level of erosion in the palatal sites is greater than in the labial or buccal sites. The properties of the surface of the substrate on which the pellicle is formed may influence the rate of formation and thickness of pellicle [Sonju and Rolla, 1973]. Hay [Hay, 1969] found that the pellicle formed on unabraded bovine enamel surfaces exhibit the same surface characteristics as other hydroxyapatites such as human enamel with regard to salivary protein adsorption.

7.2.3 The Erosion in situ design

The erosion in situ study design has been used extensively by many research groups [Hall et al., 1999; Jaeggi and Lussi, 1999] in studies to evaluate the erosive potential and the protective effects of oral health products. Due to clinical relevance, most in situ models consider both erosion and abrasion challenges. However, “pure” erosion models are important for the study of the erosion mechanisms. In situ erosion/abrasion models can associate clinical relevance test conditions with highly sensitive outcome measures. All three factors that interplays the erosion process could be controlled in an in situ study.

In these models, specimens of dental tissue are carried in the mouth and are exposed to erosive challenges or other treatment at pre-determined times, either intra-orally or ex vivo. Tissue loss is measured ex vivo, permitting the use of accurate analytical methods. In situ models thus provide a method of accurately measuring tissue loss over time in a more or less completely natural environment of saliva flow, pellicle development and routine oral care.

Meurman and Frank [Meurman and Frank, 1991a] were some of the first researchers who attempted to describe early erosion of different types of enamel (prismatic and
aprismatic human enamel, polished and unpolished bovine enamel). Using scanning
electron microscopy, they described how the various enamel appeared after 15 -180
minutes of immersion in some acidic beverages and speculated that these
ultrastructural differences modified the progression of in vitro erosion.

7.2.4 Natural and polished enamel samples

It is well established that the outer natural surfaces of enamel exhibit aprismatic areas
[Newman and Poole, 1974], with a thickness of up to 100 µm [Whittaker, 1982]. Within
this aprismatic surface enamel, there is an absence of prism boundaries and the
apatite crystals are arranged parallel to each other and perpendicular to the surface
[Robinson et al., 1995]. Hence aprismatic enamel may be more mineralised than
deeper areas. The fluoride content of the superficial enamel is also found to be higher
than the deeper areas [Nakagaki et al., 1987; Weatherell et al., 1977]. Prismatic
enamel which is present on the surface of polished samples, on the other hand, shows
abrupt changes in crystal orientation at the prism boundaries.

Meurman and Frank [Meurman and Frank, 1991a] used scanning electron microscopy
to study natural and polished bovine and natural human enamel samples. They
concluded that besides the chemical properties of an erosive agent, the ultrastructural
factors in enamel also greatly modified the progression of erosion. Polished samples
which consist of mainly prismatic enamel showed more pronounced dissolution than
natural samples and a characteristic dissolution where initial erosion after 15 min
immersion was seen to affect specifically the prism sheath areas.

To mimic intra-oral erosion as closely as possible, it is desirable to assess the effects
of erosive agents or preventive agents on natural tooth surfaces. However, most of the
tools and methods available for the assessment of erosion are design for flat polished
samples, which means that the natural, often fluoridated surface, of the tooth has to be
removed. However, in the case of active ongoing intra-oral erosion, the outermost
surface layers are also continuously removed by the acid attack and other intra-oral
mechanical forces, such as abrasion and attrition, resulting in a surface not dissimilar to that of a polished surface. Therefore, both types of the enamel substrates have a place in erosion studies depending on the objective of a study.
7.3 Objectives and Working Hypothesis

The aim of this study was to investigate the sensitivity of OCT and QLF in detecting early erosion in an *in situ* setting before progressing to using it in a true *in vivo* situation. The sensitivity of OCT and QLF were compared to the more established technique of measuring early enamel erosion using microhardness.

The primary outcomes of the study were:

1. To evaluate the sensitivity of OCT and QLF in the detection of early enamel erosion clinically

2. To evaluate the difference of response to acid challenge between natural-surface and polished surface enamel.

3. To evaluate the difference in erosion after different periods of exposure to the oral environment.

The Null Hypotheses tested were:

1. QLF is not able to detect and longitudinally measure early enamel erosion on natural- surface enamel *in situ*.

2. OCT is not able to detect and longitudinally measure early enamel erosion on natural- surface enamel *in situ*.

3. QLF is not able to detect and longitudinally measure early enamel erosion on polished- surface enamel *in situ*.

4. OCT is not able to detect and longitudinally measure early enamel erosion on polished- surface enamel *in situ*.

5. Polished-surface enamel and natural surface enamel do not erode in the same rate.

6. Pre-treatment of samples in the oral environment prior to erosion does not affect the rate of demineralisation.
7.4 Materials and Methods

7.4.1 Study design

A double-blind (blinded to the research participants and the persons responsible for performing the sample analysis) *in situ* study was carried out on healthy participants wearing appliance that were embedded with human enamel samples. The study protocol was reviewed and approved by a local ethics committee (Devon & Torbay Research Ethics Committee, reference no 09/H0202/60).

7.4.2 Enamel samples

80 intact, caries-free human incisor teeth that were recently extracted from patients aged 18 or over, of either gender were collected following ethical approval. Sample size calculation was performed based on Hooper et al’s study [Hooper et al., 2004] as described in section 7.4.3. All teeth were soaked in 20,000 ppm sodium hypochlorite to water solution for at least 24 hours as a disinfection procedure [Kohn et al., 2003], and then scraped clean of any remaining tissue with a scalpel. The teeth were then washed in distilled water. The teeth were randomly divided into 4 groups (n = 20) as described below.

Group A – Enamel surface not polished (natural surface), exposed to the oral environment for 30 days prior to acid challenge.

Group B – Enamel surface not polished (natural surface), exposed to the oral environment for 15 days prior to acid challenge.

Group C – Enamel surface not polished (natural surface), no prior exposure to the oral environment prior to acid challenge.

Group D – Enamel surface polished, no prior exposure to the oral environment prior to acid challenge.
7.4.2.1 Preparation of natural-surface enamel samples

The coronal part of the tooth was sectioned from the root using a water cooled high speed diamond saw, Microslice II (Ultratec Manufacturing Inc, Santa Ana, USA). The coronal sections were then further sectioned to an area of approximately 4 mm x 4 mm. The flattest part of the specimen (labial surface) was positioned next to the cutting blade. The blade was then retracted 3.5 mm where upon a cut was made, giving each specimen a uniformed height of 3.5 mm to allow for sufficient dentine underneath the enamel. The lingual surface was designated as the working surface. Each section was mounted in Stycast resin (Stycast; Hitek Electronic Materials, Scunthorpe, UK), with the natural surface facing upwards so as to avoid any contact with resin on the natural surface and the resin was left overnight to set.

7.4.2.2 Preparation of polished-surface enamel samples

Specimens that were polished flat were sectioned as above and mounted in resin with the natural surface face down in the mould. Once the resin had set, the specimens were removed from the mould and the backs were polished using silicon carbide discs (p1200) so that it would sit flat in a polishing jig. The labial surface was then polished flat using silicon carbide discs (p1200), followed by slurry of 3 µm silica powder in deionised water and finished with slurry of 0.3 µm alumina powder in deionised water. The specimens were ultrasonicated in deionised water in between each polishing stage to remove any powder debris.

7.4.2.3 Preparation of the enamel surface (bonding agent and laser indents)

The enamel surfaces were divided into 3 equal portions of 3 mm x 1 mm, with the middle third serving as the area to be exposed to acid challenge and the right and left thirds as the reference areas.

Due to the inherent assessment constraints of the surface microhardness method, an approximately 2mm x 1 mm area of the exposed area which is within 300µm of height variation was identified using a non-contact profilometer (Proscan2000, Scantron, UK).
These identified areas were recorded by means of measurement from the edges of the samples and three laser indents (Figure 7.1 and Figure 7.2) in triangular configuration were made to mark the area. The laser indents were made using a Er:YAG laser, Fidelis 320A (Fotona) with the following configuration: Very Short Pulse (VSP), 600milliJoule, 2 Hz for 2 seconds.

The two reference sides including the laser indents were coated with the dentine bonding agent, Prime and Bond NT, (Dentsply, UK) while leaving the centre area of approximately 1 mm (X-axis) x 3 mm (Y- axis) exposed (Figure 7.1). Between measurements, the samples were kept in a humidified Eppendorf holder.

7.4.3 Recruitment of participants

Sample size calculation was based on [Hooper et al., 2004], comparing test formulation versus negative control following 10 treatment days. The standard deviation of the difference between the benchmark paste and the negative control paste was estimated from the study and the p-value given as 2.38 (the mean difference is 1.65). A sample size of 19 will have 80% power to detect a difference in means of 1.650, assuming a standard deviation of differences of 2.380, using a paired t-test with a 0.050 two-sided significance level. Participants were screened up to one month prior to the start of study. 25 healthy participants were screened and 20 who fulfilled all the entry criteria (Table 7.1) were recruited. They consisted of 6 males and 14 females with a mean age of 24 (ranging from 20 to 35).

Prior to receiving any study specific procedures, participants were provided with Participant Information Sheet and signed an informed consent form. Personal medical history and concomitant medication information was obtained, reviewed, and retained as site source documentation. Demographic information and study entrance criteria was obtained and documented on the appropriate case report form (CRF). An oral soft tissue examination was conducted and documented on the appropriate CRF. Subjects
were issued a standard dentifrice (Colgate® Cavity Protection) and toothbrush (Colgate® Total® Professional) to be used at home for the duration of the study.

7.4.4 Intra-oral appliances
Right or left mandibular buccal customised intra-oral appliances were made for each participant. A lower alginate impression was recorded in a perforated stock tray and then poured in Kaffir D dental stone within 30 minutes and lower left or right intra-oral appliances was constructed. Cribs were constructed to fit suitable posterior teeth to aid retention. A channel was made along the buccal aspect of each appliance to accommodate one enamel sample from each of the four experimental groups (Figure 7.2). Three out of these four enamel samples were samples with untouched, natural surface samples while the fourth one was with polished surface. The positions in the appliance of the enamel samples from the different groups were randomised.

7.4.5 In situ phase of study
The study was carried out in two phases. Phase I of the study involved exposing the enamel samples in Group A and B to the oral environment for different periods of time prior to the acid challenge while Phase II is the continuation of Phase I for 5 days but with simultaneous in-vivo acid challenge. All four groups were involved in Phase II of the study (Figure 7.3). Each of the participants completed a total treatment duration of 35 days.

7.4.5.1 Phase I - Exposure of enamel samples in Group A and B to the oral environment
On Day 1 of this study phase, one enamel sample from Group A was placed randomly at one of the four sample slots of the intra-oral appliance, as assigned by a random table generated using SPSS v 16.0.

The participants were then instructed to wear the intra-oral appliance from 9:00 +/- 30 minutes to 16:30 +/- 30 minutes, except for a 1 hour period over lunch resulting in each
subject wearing the appliances for a minimum of 6 hours daily. The appliances were returned to the study site at 16:30 ± 30 minutes each day and stored in a ‘moist pot’ containing a damp cotton wool pad overnight. Participants were issued a standard dentifrice (Colgate® Cavity Protection) and toothbrush (Colgate® Total® Professional) to use at home for the whole duration of the study.

On Day 16, one sample from Group B was added to the appliance, positioned as assigned by a random table as above. This daily protocol was then repeated for an additional 15 days.

At the start and end of each study day, the intra-oral appliances were disinfected with 70% ethanol spray, left for 2 minutes and then rinsed under running water. A soft bristle toothbrush was used to remove any debris and plaque formation on the appliances and samples.

At the end of Day 30, following disinfection, the enamel samples were removed from the appliances and baseline measurement taken using surface microhardness, quantitative light fluorescence (QLF) and optical coherence tomography (OCT). After measurements were taken, the enamel samples were placed back into the intra-oral appliances and kept in the moist pots as noted above.

7.4.5.2 Phase II - Acid challenge of all study groups

On Day 31, one sample each from Group C and Group D was added to the remaining two empty two slots, positioned as assigned by the generated random table noted above. During this phase of study the participants continued to wear the intra-oral appliance from 9:00 +/- 30 minutes to 16:30 +/- 30 minutes, except for a 1 hour period over lunch as per Phase I of the study.

In addition to that, there were three in-vivo acid challenges daily at 9:00 +/- 30 minutes, 13:00 +/- 30 minutes and 15:30 +/- 30 minutes which were undertaken under the supervision of a member of the study site staff. During these in vivo acid challenges,
the participants were asked to orally swish with 25ml of orange juice, Sainsbury’s Smooth Orange Juice from Concentrate (Sainsbury’s Supermarkets Ltd, 33 Holborn, London EC1N 2HT) (pH = 3.4 ± 0.1), for 1 minute, expectorate, and then swish with a fresh 25ml of orange juice again. This was repeated until participants have swished with 25ml of orange juice 10 consecutive times, thereby exposing the enamel samples to 250 ml of the acidic beverage over a 10 minute period. A total of 750ml will be swished around the mouth for a total of 30 minutes each day, on alternate days, over a 2 week period.

7.4.6 Measurement of samples

The appliances were returned to the study site at least 1 hour after the last acid challenge of each day. Prior to performing any measurements on the enamel samples, the appliance were sprayed with a 70% ethanol solution, left for 2 minutes and then rinsed under running water. Instead of using a soft bristle toothbrush to remove any debris and plaque, as was done during Phase I of the study, the surface of the enamel sample were wiped with 0.2% Chlorhexidine Gluconate [Azzopardi et al., 2004] using cotton pellets. This was necessary to avoid tampering with the softened eroded surfaces.

Following disinfection, the enamel samples were removed from the appliance and placed in humidified Eppendorf receptacles prior to being measured using surface microhardness, quantitative light fluorescence (QLF) and optical coherence tomography (OCT).

7.4.6.1 Surface microhardness

The micro indenter, Microhardness Tester FM-700; Future-Tech Corporation, Japan was used in the study. A triple charged coupled device (3CCD)-colour camera of 1014 x 768 pixel resolution (HV-F31, Hitachi Kukasai Electric U.K. Ltd) was attached to the micro indenter and was operated via bespoke software.
The resin blocks with embedded enamel samples were placed flat on the translation stage and fixed at a reproducible position with the precision vice of the micro indenter. A surface area of approximately 1 mm x 1 mm, perpendicular to the direction of the load of the indenter was identified at the uncoated middle third of the samples for indentation. The identification of the perpendicular area was achieved by ensuring that the aiming beam of the micro-indenter was in focus before loading was performed. Micro indents were made using a Knoop diamond indenter, with a load of 25 g applied for 5 seconds.

At the identified area, 5 indentations approximately 100 µm apart were made during each measurement time point. Each indent made was observed and imaged under 50 x magnifications.

Although care was taken to ensure that indentations were carried out on a surface that was perpendicular to the direction of loading, due to the inherent curvature of a natural and unpolished enamel surface and the presence of local irregularities such as perikymata, some severely asymmetrical indents were observed and were not included in the analysis. In these cases, additional indents were made.

A program was written in MATLAB (The MathWorks Inc, USA) to measure the indents. The person performing the measurement of the indents was blinded to the session of acid challenge of the indents imaged. Due to the inherent natural curvature of the surface enamel, some of the sides and points (angles) of the rhomboidal-shaped indents were not apparent / obvious, resulting in asymmetrical looking indents. On these occasions, the program was used together with a screen protractor to measure the imaged indents from the various different measurement time points. A rhomboidal-shaped screen overlay with the dimension of the indent of the Knoop indenter (longitudinal angle of 172.50° and transverse angle of 130 °) [Knoop et al., 1939] was built using a screen protractor, MB-Ruler 4.0 (MB-Softwaresolutions). This overlay was
placed over asymmetrical indents in order to be able to project the length of the horizontal diagonal of the indentations.

The horizontal lengths of the 5 indents were averaged and the Knoop numbers ($KHN$) were calculated. The outcome measure was expressed as the percentage of surface microhardness change ($ΔSMC$), calculated based on the differences between Knoop hardness numbers at baseline, $KHN(t_0)$ and the subsequent erosion intervals, $KHN(t)$. $ΔSMC$ was calculated as:

$$ΔSMC(t) = 100 \left( \frac{KHN(t) - KHN(t_0)}{KHN(t_0)} \right)$$

7.4.6.2 QLF

The same QLF set up used described in Chapter 5 and 6 were used in this study.

Samples removed from the Eppendorf receptacles were dried under compressed air for 20 seconds. A customised mount for the enamel samples was placed in a dark enclosure and QLF images of the samples were taken in this enclosure. The images were captured with bespoke software that enabled video repositioning of the samples (where there is a semi-automated alignment of images) from different measurement time points. Optimum light intensities and camera settings were determined to ensure that the fluorescence images from the sample were within the detection dynamic range of the camera and of maximum contrast.

The program written in MATLAB (The MathWorks Inc, USA) described in Chapter 5 and 6 for analysing data of the in vitro study was modified to manage two reference areas instead of one. Images from each specimen at the various erosion intervals were aligned so that representative regions of interest (ROI) of the same location and dimension throughout the erosion intervals for the exposed and non-exposed area could be drawn.
The mean pixel value of the green channel was obtained for the defined ROI for the exposed, $F_E(t)$, and non-exposed $F_{NE}(t)$, areas. All images were analysed blind to the erosive challenge intervals, $t$. The percentage loss of fluorescence of the exposed area at each erosion interval, $\Delta F(t)$, was calculated as follows:

$$\Delta F(t) = 100 \left[ \frac{F_E(t)}{F_E(t_0)} - \frac{F_{NE}(t)}{F_{NE}(t_0)} \right]$$

where $t_0$ is the baseline time point.

**7.4.6.3 OCT**

The OCS1300SS OCT system (Thorlabs Ltd, UK) was used to capture three dimensional OCT data from the exposed area of enamel samples. The OCS1300SS uses Fourier Domain technology and incorporates a broadband, frequency swept laser which was centred at 1325 µm. It has an axial resolution of 9 µm and transverse resolution of 15 µm in air.

The hand probe was mounted with the laser beam facing downwards and a customised mount for the enamel samples was positioned perpendicularly below the hand probe (Figure 7.4). Samples removed from the Eppendorf receptacles were dried under compressed air for 20 seconds. Enamel samples with natural surfaces were slid flat in the trough of the mount while the samples with the polished surface were placed at an angle in the trough. This was necessary for the samples with polished surface to minimise surface specular reflection. This mount provided a definite stop for the repositioning of the samples during the various measurement time points. The Thorlabs Swept Source OCT software (Thorlabs Ltd, UK) was used to control the laser to scan a 3.5 mm (X axis) x 0.5 mm (Y-axis) area of the enamel sample. The position of the laser beam was driven using an inbuilt X-Y galvanometer. The 3.5 mm (X axis) section includes the 1 mm exposed area in the centre, flanked by the non-exposed areas on either side (Figure 7.5). The optimal location of the 0.5 mm (Y-axis) in the exposed 3 mm was determined during baseline measurement. The choice of the position was
based on an area with the least specular reflection. The X-Y coordinate of the laser beam for each specimen as shown in the Thorlabs Swept Source OCT software was recorded and used for repositioning of the laser beam in subsequent measurement time periods.

The distance of the hand probe from the surface of the specimen was determined with the most convex area of the labial surface of the tooth at 1.0 ± 0.1 mm from the top of the display window of the image capture software. The transverse resolution of the X-axis was set at 1024 pixels in 3.5mm and Y-axis at 24 pixels in 0.5 mm. The axial resolution of the Z-axis was set 512 pixels in 2.01mm.

The maximum amplitude was calibrated with an acrylic sample after the measurement of every eight samples. The dynamic range of the OCT light was maintained to be around 20 – 30 decibels (dB), as suggested by Baumgartner et al [Baumgartner et al., 2000]. Background noise was removed before the acquisition of each image [Schoenenberger et al., 1998]. The power output of the laser was also monitored and recorded after every 8 measurements using a power meter (Figure 7.4).

The program written in MATLAB (The MathWorks Inc, USA) described in Chapter 5 and 6 for analysing data of the in vitro study was expanded to analyse the three dimensional data. Provisions were added to allow simultaneous cross-sectional (B-Scans) and en-face views (C-Scans) of the scanned data (Figure 7.6) and to threshold out the confounding signals from specular reflection. The C-scans of each sample from the different measuring time points were aligned and a similar region of interest of the exposed area was selected for all the six measuring time points.

Although the location of the 0.5 mm area of the Y-axis was carefully chosen to minimise the inclusion of specular reflection, it was not possible to avoid it completely. This specular reflection is particularly strong for normal incidence and the intensity varies markedly with angle of incidence. Fried et al found that the intensity in this
region is approximately 20dB higher than the scattering intensity in other areas and therefore masks any information about scattering at or just below the tooth surface. Hence they implemented a threshold level to their data [Fried et al., 2002]. For this study, the selected regions of interest on the C-scans were projected onto the respective B-scans for the determination of the threshold levels (Figure 7.7). The threshold level was adjusted until all A-scans with columnar artefacts resulting from specular reflection were removed (Figure 7.8). The final selected region of interest consisted of 8000 ± 300 of A-scans. The curvature of the tooth surface in the selected region of interest was compensated for by aligning the peak of the backscattered intensity rise occurring at the enamel-air interface in each A-scan along the same horizontal pixel line of the B-scan. A mean A-scan was then generated from the selected 8000 ± 300 A-scans for the exposed area.

\[ D, \text{ is the relationship of the attenuation of backscattered light between two optical depths of an OCT A-Scan and is represented by the function below,} \]

\[ D(t) = \frac{I_{\text{plateau}(t)}}{I_{\text{superficial}(t)}} \]

\[ I_{\text{superficial}} \text{ is the intensity of backscattered light at or immediately below the enamel-air interface where demineralisation had occurred and } I_{\text{plateau}} \text{ is the backscattered intensity where the A-scan had reached a plateau and is assumed to be unaffected by the erosive challenge (Figure 5.3). The chosen superficial optical depth for this study was 30µm below the tooth air interface and the level of plateau was observed to be 150 µm below tooth-air interface as this combination was shown to be the most sensitive in the detection and assessment of early erosion.} \]

As shown in Chapter 6, if the output of the incident light was monitored and maintained at a constant intensity throughout a study period, the decay of backscattered light of the exposed area \( D_E \), could be an acceptable outcome measure for OCT, without
needing to take into account the decay of the backscattered light of the non-exposed area.

In order to be comparable to with the calculation of the outcome measure of the other two instruments, the percentage of change in decay of the exposed area during a particular erosion interval, $\Delta D(t)$ from baseline, $D_E(t_0)$ was used. $\Delta D(t)$ for each measurement time point is represented by the function shown below:

$$\Delta D(t) = 100 \left[ \frac{D_E(t) - D_E(t_0)}{D_E(t_0)} \right]$$

where $D_E$ is $D$ of the exposed area, $t_0$ is the baseline time point and $t$, is the specific measurement time point.

### 7.4.7 Statistical Analysis

The STATA™ 10.1 (Statacorp, Texas) statistical program was used. Multiple linear regression analyses were performed, taking into account the clustering of samples using robust variance estimates, to ascertain whether erosion-interval (time) related changes were detected with the three different instruments in situ for all the four experimental groups.

When a significant time-related change was found for an outcome measure, paired t-test was performed to identify the detection sensitivity, which is defined as the shortest subsequent time intervals when a significant difference was detected.

Comparison of the regression coefficient was used to compare the rate of time related changes between the four experimental groups.
7.5 Results

7.5.1 Surface microhardness

7.5.1.1 Natural – surfaced samples

Multiple regression analysis of $\Delta SM$ with erosion interval for all these three groups (natural surface) showed that surface microhardness detected a significant ($P < 0.05$) erosion-interval related decrease, hence erosion-interval is a significant co-variate for the decrease of surface microhardness. Table 7.2 presents the results of the analysis. All three groups demonstrated negative relationship with erosion interval and Group B demonstrated the highest $R^2$ value of 0.347.

Figure 7.9 is the line graph that shows the progression of $\Delta SM$ during the study period for all four groups. At the end of the study period, Group A, which was exposed to the oral environment for 4 weeks prior to acid challenge demonstrated less cumulative decrease of microhardness ($32.70 \pm 6.24\%$) compared to Group B ($49.42 \pm 4.30\%$) and Group C ($46.03 \pm 5.05\%$). The regression coefficients of these three groups were compared to assess whether the slope of the erosion-interval related decrease were significantly different. Although Group A showed less decrease of surface microhardness compared to the other two groups of natural surface specimens, it was found that the regression coefficients of these three groups were not significantly different ($P > 0.05$) from each other.

Paired t test with a 0.050 two-sided significance level were used to analyse $\Delta SM$ between each consecutive interval of erosion for these three groups and the results are shown in Table 7.3. Surface microhardness detected significant differences between the measurements of Baseline and Day 1 of Phase II for all three groups. Subsequently, significant differences were detected between Day 1 and Day 2 for Group C, Day 1 and Day 3 for Group B and Day 1 and Day 4 for Group A. While the
ΔSMC for Group A and B did not show any more significant difference thereafter, ΔSMC for Group C between Day 3 and Day 5 were significantly different.

7.5.1.2 Polished-surfaced

Multiple regression analysis of ΔSMC with erosion interval for the polished-surface group detected significant (P < 0.05) decrease with a R² value of 0.595 (Error! Reference source not found. Table 7.2). This group, as shown in (Figure 7.9), demonstrated a faster rate of surface microhardness decrease as compared to the natural-surfaced groups, with a cumulative decrease of 81.05 ± 2.66 % at the end of the 150 minutes of erosive challenge.

The regression coefficients of Group C and Group D were compared and it was found that the regression coefficients of these two groups were significantly different (P < 0.05) from each other.

Paired t-tests of ΔSMC between each consecutive interval of erosion were also performed and significant differences were detected during the first three days and the final day of Phase II of the study (Table 7.3).

7.5.2 QLF

7.5.2.1 Natural – surfaced samples

Multiple regression analysis of ΔF with erosion interval for all these three groups (natural surface) showed that QLF detected significant (P < 0.05) erosion-interval related change of fluorescence. Hence erosion-interval is a significant co-variate for the changes in loss of fluorescence. Table 7.4 presents the results of the analysis. Group A and B demonstrated positive relationships with erosion interval while Group C demonstrated a negative relationship with erosion interval.

Figure 7.10 is the line graph that shows the progression of ΔF throughout the study period for all four groups. Group C demonstrated a loss of fluorescence with most of
the loss occurring on Day 1 of the study (\(-3.27 \pm 0.72\) %) and reaching (\(-4.18 \pm 0.90\) %) on Day 5. However, Group A and B which were exposed to the oral environment for 4 weeks and 2 weeks respectively prior to acid challenge demonstrated an increase of fluorescence throughout the study period reaching 3.09 \(\pm\) 0.51 % for Group A and 1.81 \(\pm\) 0.52% for Group B. The regression coefficients of Group A and B were compared and it was found that the slope of the erosion-interval related increase in fluorescence in these two groups were not significantly different (\(P = 0.072\)). Comparison of the regression coefficients between these two groups that were exposed to the oral environment with that of Group C were not performed as the trends in fluorescence change were obviously different.

Paired t tests with a 0.050 two-sided significance level were used to analyse \(\Delta F\) between each consecutive interval of erosion for Group C and it was found that only \(\Delta F\) between Baseline and Day 1 and between Day 1 and Day 4 differ significantly (Table 7.5).

7.5.2.2 Polished-surfaced

Multiple regression analysis of \(\Delta F\) with erosion interval for the polished-surface group detected significant (\(P < 0.05\)) loss of fluorescence \(R^2\) value of 0.072 when the pre-erosion measurement was taken as baseline (Table 7.4). As with Group C, Group D demonstrated a net negative relationship with erosion interval but there was less loss of fluorescence compared to Group C, with a cumulative loss of 1.17 \(\pm\) 0.60 % at the end of the 150 minutes of erosive challenge (Figure 7.10). It was observed that there was an initial increase in fluorescence in Day 1 but loss of fluorescence was observed thereafter. If Day 1 measurement was taken as baseline, a higher \(R^2\) value of 0.217 was demonstrated.

The regression coefficients of Group C and Group D were compared and it was found that the regression coefficients of these two groups were not significantly different (\(P=0.203\)) from each other.
Paired t-tests of $\Delta F$ between each consecutive interval of erosion were also performed and significant differences were detected between Day 1 and Day 2 and between Day 2 and Day 4 of erosive challenge as shown in Table 7.5.

7.5.3 OCT

7.5.3.1 Natural – surfaced samples
Multiple regression analysis of $\Delta D$ with erosion interval of these three groups (natural surface) showed that significant erosion interval related decrease of backscattered intensity decay was observed only in Group C (P=0.010) with a $R^2$ value of 0.072. OCT did not detect any significant erosion-interval related changes in Group A and B (P> 0.05). Table 7.6 presents the summary of the regression analysis results.

Figure 7.11 is a line graph that shows the progression of $\Delta D$ during the study period for all four groups. Group C reached its maximum decrease in backscattered decay of 11.87 ± 3.51% by Day 2 of erosive challenge. Paired t- test of $\Delta D$ between each consecutive interval of erosion showed that there were significant changes between Baseline and Day 1 and between Day 1 and Day 2. (Table 7.7)

7.5.3.2 Polished-surfaced
Multiple regression analysis of $\Delta D$ with erosion interval of polished surface group showed significant erosion-interval related decreases of backscattered intensity decay in Group D (P< 0.000) with a $R^2$ value of 0.3010 (Table 7.6).

As shown in Figure 7.11, the decay of backscattered intensity decreased markedly during the study period. By Day 3 of erosive challenge, there was a mean decrease of 34.71 ± 5.63 and reaching 40.57 ± 5.58 % at the end of the study. Paired t- test of $\Delta D$ between each consecutive interval of erosion showed that there were significant changes on each day of erosive challenge until Day 3 (Table 7.7).
7.6 Discussion

7.6.1 *In situ* study design

The main biological and chemical conditions related to dental erosion were reproduced in this *in situ* model, with the natural formation of salivary pellicle, and the presence of physiologically secreted saliva during the orange-juice-induced erosive challenge. The choice of a two-hour pellicle was made based on the equilibrium between protein adsorption and de-sorption being reached within 2 hrs [Lendenmann et al., 2000] and it has been shown that a two-hour pellicle is acid-resistant [Meurman and Frank, 1991b; Sonju Clasen et al., 1997].

The lower buccal appliance was the location of choice so as to minimise soft tissue wear from the tongue. A previous study has shown the high wear effect of the tongue on hard tissue samples carried with palatal appliances [Azzopardi et al., 2004].

7.6.2 Surface microhardness

The QLF and OCT measurements in this *in situ* study were once again validated against surface microhardness as what was done for the *in vitro* data (Chapter 5). Although different types of teeth were used, the same load and dwelling time were used as a preliminary evaluation showed that this combination produced the smallest discernible indentations, both for the natural and polished samples under 50 x magnifications.

When comparing the measurements of indents between Group C (natural surface) and Group D (polished surface), it is observed that the standard errors of the measurements on natural surfaces were considerably higher than those on polished surfaces during the last two days of erosive challenge. This is illustrated in Figure 7.9. The reason for this could be multifactorial. One of the reasons was higher measurement error caused by the inherent difficulty of measuring indents on natural surfaces and also as indents become larger with erosion interval, it becomes more
challenging to obtain symmetrical indents. In addition to the above reasons, it could also be due to the different manner of dissolution of these two different substrates. Human aprismatic surface enamel has been reported to exhibit an irregular type of dissolution with severely affected areas adjacent to less affected ones, while human prismatic enamel showed a more characteristic uniform dissolution of the prism sheath areas during the initial 15 minutes of immersion in Cola beverage or acidic sports drink. It is then followed by dissolution of enamel prism cores and finally the interprismatic areas in longer immersion periods. [Meurman and Frank, 1991a].

Similar to what was observed in the in vitro study (Chapter 5), the decrease in percentage of surface microhardness with erosion time appears to follow more of an exponential function than a linear one. As shown in Figure 7.9, this trend is much more prominent in the polished - surface group than the natural – surface group. This is in agreement with Lippert et al’s and Zheng et al’s [Lippert et al., 2004; Zheng et al., 2009] work. Davidson and co workers observed that the mechanical properties of enamel, like density, and modulus of elasticity and microhardness, drop sharply in the initial phases of demineralisation [Davidson et al., 1974]. This observation was postulated to be due to partial transformation of the hydroxyapatite crystals into calcium phosphate, which has similar chemical composition but different physical properties [Groeneveld et al., 1974].

The multiple linear regression results showed that the decrease in surface microhardness between the three natural surface groups did not differ significantly. This indicated within the confines of the design of the current study, exposing the enamel samples to the oral environment prior to the erosive challenge did not increase the resistance of the natural surfaced samples. However, the decrease of $\Delta SMC$ in Groups A and B showed signs of slowing down from Day 4 onwards (Figure 7.9) as attested by the results of the paired t-test where no significant difference were detected after Day 4 for Group A and Day 3 for Group B, whilst the decrease of $\Delta SMC$ of Group
C were significant up to final day of the study. If the trends of decrease in Group C were to be projected further along the erosion interval axis, further decrease of $\Delta SM C$ could be expected but this would have to be verified in further studies.

When comparing the $\Delta SM C$ of the natural surface and polished surface groups, it is apparent that the $\Delta SM C$ of the polished group were much more extensive and this is in agreement with previous work that reported higher erosion rate in polished samples when compared to natural samples [Ganss et al., 2000; Meurman and Frank, 1991a].

7.6.3 QLF

The outcome measure, $\Delta F$, is the percentage change of fluorescence of the exposed area relative to the non-exposed area. An assumption was made that no fluorescence change was to be expected from the non-exposed area during the erosive challenge period other than that resulting from fluctuations of the incident light. Measures were taken to ensure that the energy output of the incident light was kept constant throughout the study, as described in section 7.4.6.2.

A decrease of this outcome measure was to be expected from the exposed area after an erosive challenge since demineralisation occurs. However, the contrary was observed for Groups A and B. Such increase of $\Delta F$ could be due to either a true increase of fluorescence of the exposed area or a higher loss of fluorescence of the non-exposed area relative to the exposed area. The latter is the more likely reason as surface microhardness measurements (as presented in Sections 7.5.1.1 and 7.5.1.2 and discussed in Sections 7.6.2) had shown that demineralisation did indeed occur.

Pretty et al in 2001 [Pretty et al., 2001] showed that the change of fluorescence of QLF are susceptible to the presence of stains and this could be used to longitudinally quantify stains. Amaechi and Higham [Amaechi and Higham, 2002] subsequently found that the detection and longitudinal monitoring of incipient caries with QLF may be
limited by the presence of saliva or plaque and enhanced by staining. Although the participants of this present study were instructed to refrain from consuming drinks or food that could potentially stain the specimens, the surfaces especially the non-exposed area could still potentially be stained by the orange juice, as well as by the 0.2 % chlorhexidene gluconate [Hofer et al., 2011] that was used to wipe the specimens at the end of each day during Phase II of the study.

The dentin bonding agent applied on the non-exposed areas of specimens from Group A and B were exposed to the oral environment prior to being subjected to the erosive challenge. There might have been some degradation of the surface roughness of the dentine bonding agent, with its exposure in the oral environment due to organic breakdown and mechanical wear from the buccal mucosa. It had been reported previously that dentine bonding agent were susceptible to chemical, as well as mechanical degradation. Chemically, the most important reactions are hydrolysis and plasticizing of the resin components, which are both related to the ingression of water [Sano et al., 1995]. Increased surface roughness will cause an increase uptake of stains which in turn causes loss of fluorescence. The rate of loss of fluorescence caused by the staining of the non-exposed area could have exceeded the rate of loss of fluorescence due to demineralisation at the exposed area, resulting in a net gain of fluorescence based upon the formula for $\Delta F$. As for Group C, there was an initial big loss of fluorescence (-3.27 ± 0.72 %) on Day 1 of the erosive challenge but no significant loss of fluorescence was detected thereafter. The initial quantum loss of fluorescence observed mirrors the initial sharp changes observed in other mechanical properties like modulus elasticity and surface microhardness [Lippert et al., 2004; Zheng et al., 2009] during demineralisation. Any loss of fluorescence of the exposed area due to demineralisation thereafter could similarly be masked by the increase of stain uptake of the non-exposed areas but the rate of uptake of stains may be less than those occurring in Group A and B.
\( \Delta F \) for Group D demonstrated an initial increase in fluorescence in Day 1 (Figure 7.10) but results of the paired t test showed it was not significantly different from baseline. There was loss of fluorescence thereafter. As shown by the surface microhardness results, the rate of demineralisation of this group is higher than specimens in the natural surfaced groups, therefore greater loss of fluorescence of the exposed area due to demineralisation would be expected from this group. This greater loss of fluorescence results in the observed net loss of fluorescence.

### 7.6.4 OCT

The outcome measures for surface microhardness (\( \Delta SMC \)) and OCT (\( \Delta D \)) were calculated without using the non exposed areas. Therefore they are not confounded by any possible changes to the non-exposed areas, such as staining or increased surface roughness.

The trends of changes of backscattered intensity decay (\( \Delta D \)) for Group C and D are similar to the decrease of surface microhardness of the similar groups with the decrease in Group C less than that of Group D. However, the decrease of surface microhardness in Groups A and B were not reflected by similar changes in \( \Delta D \). This phenomenon could be related to the masking of the backscattered intensity by the presence of mature pellicle on the exposed area.

### 7.6.5 Comparison of results of in vitro and in situ study

Statistical comparison of the in vitro studies presented in Chapters 5 and 6 with that of this study could not be done as they were separate studies conducted in separate occasions with different instrument configurations and acidic challenges conditions. Nevertheless, as found by other researchers [Hall et al., 1999], in vitro results demonstrated a positive correlation with in situ results.

Surface microhardness in the vitro study (Chapter 5) detected significant erosion-interval related decrease in microhardness, with a mean 81.40 ± 2.70 % at the end of
the 60 minutes *in vitro* erosive challenge, whilst the mean ΔSMC of Group C in this study was 46.03 ± 5.05% at the end of 150 minutes *in vivo* erosive challenge.

These observations are in agreement with other previous studies that showed *in vitro* erosive challenge is more severe than *in vivo* challenges in the absence of the buffering effects of saliva. Hence, the sensitivity of assessment tools needs to be increased in order to be used in *in vivo* studies or the interval of the erosive challenges need to be extended.

### 7.7 Conclusions

1. OCT and QLF were able to detect and longitudinally measure *in situ* demineralisation in natural samples subjected to 150 minutes of *in vivo* acid challenge with orange juice.

2. OCT and QLF were able to detect and longitudinally measure *in situ* demineralisation in polished samples subjected to 150 minutes of *in vivo* acid challenge with orange juice.

3. Polished surfaces eroded faster than natural surfaces in the conditions of the study.

4. Pre-treatment of samples in the oral environment prior to erosion does not affect the rate of demineralisation.
7.8 References


### Table 7.1
Table of Inclusion and Exclusion criteria for the selection of study participants

<table>
<thead>
<tr>
<th>Inclusion Criteria</th>
<th>Exclusion Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Demonstrates understanding of the study and willingness to participate as evidenced by voluntary written informed consent and has received a signed and dated copy of the informed consent form.</td>
<td>Current or relevant previous history of serious, severe or unstable physical or psychiatric illness, or any medical disorder that may require treatment or make the subject unlikely to fully complete the study, or any condition that presents undue risk from the study products or procedures.</td>
</tr>
<tr>
<td>Aged at least 18 years.</td>
<td>Diabetes Mellitus.</td>
</tr>
<tr>
<td>Understands and is willing, able and likely to comply with all study procedures and restrictions.</td>
<td>Allergy/Intolerance - Known or suspected intolerance or hypersensitivity to the study materials (or closely related compounds) or any of their stated ingredients.</td>
</tr>
<tr>
<td>Good general health with no clinically significant and relevant abnormalities of medical history or oral examination.</td>
<td>Women who are known to be pregnant or who are intending to become pregnant over the duration of the study.</td>
</tr>
<tr>
<td>Good oral health and be able to accommodate the lower bi-lateral buccal intra-oral appliances each fitted with up to four enamel samples</td>
<td>Women who are breast-feeding.</td>
</tr>
<tr>
<td></td>
<td>Susceptibility to acid regurgitation.</td>
</tr>
<tr>
<td></td>
<td>Any condition or medication that causes Xerostomia.</td>
</tr>
<tr>
<td></td>
<td>Any orthodontic appliances, restorations, bridgework or dentures that would interfere with the study evaluations.</td>
</tr>
<tr>
<td></td>
<td>Presence of current or recurrent oral disease pathology that could affect the assessments for example aphthous ulcer and extensive carious lesions.</td>
</tr>
<tr>
<td></td>
<td>Excessive gingival inflammation.</td>
</tr>
<tr>
<td></td>
<td>An employee of the sponsor, member of the study site or a staff family relative. The site for this protocol is the Clinical Trials Unit in the Bristol Dental School and Hospital. Employees of the Bristol Dental School and Hospital not associated with the Clinical Trials Unit are eligible to participate.</td>
</tr>
</tbody>
</table>
Table 7.2

Results of Multiple Regression Analysis of percentage of surface microhardness change, $\Delta SM C$ and Erosion Interval for the 4 experimental groups.

<table>
<thead>
<tr>
<th>Group</th>
<th>Coefficient</th>
<th>$R^2$</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>-0.0601</td>
<td>0.2058</td>
<td>&lt; 0.000</td>
</tr>
<tr>
<td>B</td>
<td>-0.0826</td>
<td>0.3465</td>
<td>&lt; 0.000</td>
</tr>
<tr>
<td>C</td>
<td>-0.0784</td>
<td>0.1844</td>
<td>&lt; 0.000</td>
</tr>
<tr>
<td>D</td>
<td>-0.1392</td>
<td>0.5945</td>
<td>&lt; 0.000</td>
</tr>
</tbody>
</table>

$\Delta SM C$ of all four groups demonstrated significant negative relationship with erosion interval. $\Delta SM C$ of Group D (Polished-surface) demonstrated the highest $R^2$ value of 0.59.
Table 7.3

Paired t test results of ΔSMC for all four groups.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
<th>Day 4</th>
<th>Day 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>√&lt; 0.000</td>
<td></td>
<td>√0.002</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>√&lt; 0.000</td>
<td></td>
<td>√0.005</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>√0.009</td>
<td>√0.026</td>
<td>√0.007</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>√&lt; 0.000</td>
<td>√&lt; 0.000</td>
<td>√&lt; 0.000</td>
<td>√0.003</td>
<td></td>
</tr>
</tbody>
</table>

‘√’ represents the detection of significant difference for the indicated interval and the P values are given below the ‘√’ sign. The cells shaded grey indicates no significant difference was detected for that interval.

Surface microhardness detected significant differences between the measurements of Baseline and Day 1 for all four groups. Thereafter, the period where significant differences were detected varied among the groups with Group D demonstrating the most number of intervals with significant differences.
Table 7.4

Results of Multiple Regression Analysis of Percentage Loss of Fluorescence, $\Delta F$ and Erosion Interval for the 4 experimental groups.

<table>
<thead>
<tr>
<th>Group</th>
<th>Coefficient</th>
<th>$R^2$</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0.5110</td>
<td>0.1234</td>
<td>$&lt; 0.000$</td>
</tr>
<tr>
<td>B</td>
<td>0.2960</td>
<td>0.0639</td>
<td>0.002</td>
</tr>
<tr>
<td>C</td>
<td>-0.6400</td>
<td>0.1079</td>
<td>0.003</td>
</tr>
<tr>
<td>D</td>
<td>-0.5260</td>
<td>0.0720</td>
<td>$&lt; 0.000$</td>
</tr>
</tbody>
</table>

$\Delta F$ of Group A and B demonstrated significant positive relationship with erosion interval while those of Group C and D demonstrated significant negative relationship.
Table 7.5

Paired t test results of $\Delta F$ for Group C and D

<table>
<thead>
<tr>
<th>Groups</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
<th>Day 4</th>
<th>Day 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>✓</td>
<td></td>
<td>✓</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.001</td>
<td></td>
<td>0.021</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D</td>
<td></td>
<td>✓</td>
<td>✓</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.002</td>
<td>0.002</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

‘✓’ represents the detection of significant difference for the indicated interval and the P values are given below the ‘✓’ sign. The cells shaded grey indicates no significant difference was detected for that interval.

For Group C, it was found that only $\Delta F$ between Baseline and Day 1 and between Day 1 and Day 4 differ significantly. For Group D, significant differences of $\Delta F$ were detected between Day 1 and Day 2 and between Day 2 and Day 4 of erosive challenge.
Table 7.6

Results of Multiple Regression Analysis of Percentage of Change in Decay of OCT Backscattered Intensity, $ΔD$ and Erosion Interval for the 4 experimental groups.

<table>
<thead>
<tr>
<th>Group</th>
<th>Coefficient</th>
<th>$R^2$</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0.0159</td>
<td>0.0172</td>
<td>0.064</td>
</tr>
<tr>
<td>B</td>
<td>0.0001</td>
<td>0.0000</td>
<td>0.991</td>
</tr>
<tr>
<td>C</td>
<td>-0.0171</td>
<td>0.0722</td>
<td>0.010</td>
</tr>
<tr>
<td>D</td>
<td>-0.0800</td>
<td>0.3010</td>
<td>$&lt;0.000$</td>
</tr>
</tbody>
</table>

Significant erosion interval related decrease of backscattered intensity decay was observed only in Group C ($P=0.010$) and Group D ($P<0.000$).
Table 7.7

Paired t test results of $\Delta D$ for all four groups.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
<th>Day 4</th>
<th>Day 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>$\checkmark$ 0.001</td>
<td>$\checkmark$ 0.012</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>$\checkmark$ 0.001</td>
<td>$\checkmark$ &lt;0.000</td>
<td>$\checkmark$ 0.004</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$'\checkmark'$ represents the detection of significant difference for the indicated interval and the P values are given below the $'\checkmark'$ sign. The cells shaded grey indicates no significant difference was detected for that interval.

For Group C, it was found that only $\Delta D$ between Baseline and Day 1 differ significantly. For Group D, significant differences of $\Delta D$ were detected every day until Day 3 of the erosive challenge.
Figure 7.1

QLF image of a natural-surface enamel sample embedded in acrylic. The exposed area (A) was flanked by dentine bonding agent covered areas (B) on either side. The three laser indents (C) in a triangular configuration are also evident.
Figure 7.2

Figure shows the intra-oral appliance (A) that was used to deliver the acrylic-embedded enamel samples (B) into the mouth. Inset (C) is a close up of the enamel sample showing the three laser indents in triangular configuration.
Figure 7.3

Flow chart of the study.

- Oral appliances carrying Group A enamel samples introduced into the oral environment
- Enamel samples in Group B added to the intra oral appliances
- Baseline measurement of all four groups
- Enamel samples in Group C and D added to the intra oral appliance
- In vivo acid challenge
- Ex vivo measurement of all four groups at the end of each day.
Figure 7.4

Figure shows the OCT set-up. The OCT hand probe (B) is set with the laser beam directed downwards and perpendicular to the sample mount (D). For every 8 measurements, the power output of the laser is monitored and recorded with a power meter (A). The laser power detector (C) was placed at a fixed position from the hand probe when a reading was taken.
Figure 7.5

Figure shows the dimensions of an OCT C-scan. * indicates the 1mm exposed area and ** indicates part of a laser indent.
Figure 7.6

Figure shows the graphic user interface of the OCT analysis program.
Figure 7.7

Figure shows the C- and B-scan of a natural-surface sample. A – C-Scan of a natural surfaced sample; B - B-scan of the slice indicated by the dashed line on the C-Scan above; C - Columnar artefact resulting from specular reflection; D – The Region of Interest (ROI) drawn on the middle exposed area.
Figure 7.8

Figure shows the C- and B-scan of a natural-surface sample shown in Figure 7.7 after threshold is in place. A – C-Scan of a natural surfaced sample; B - B-scan of the slice indicated by the dashed line on the C-Scan above; C - Columnar artefact removed after thresholding; D – The Region of Interest (ROI) drawn on the middle exposed area.
At the end of the study period, among the natural surface groups, Group A, demonstrated less cumulative decrease of microhardness (32.70 ± 6.24%) compared to Group B (49.42 ± 4.30%) and Group C (46.03 ± 5.05%). Group D however, demonstrated a faster rate of surface microhardness decrease as compared to the natural-surfaced groups, with a cumulative decrease of 81.05 ± 2.66 % at the end of the 150 minutes of erosive challenge.
Graphs show the progression of the percentage of loss of fluorescence ($\Delta F$) with erosion interval throughout the study period.

Group C and D, which were not subjected to the oral environment prior to the Phase II of the study, demonstrated net loss of fluorescence as erosive challenges progressed. However, Group A and B which were exposed to the oral environment for 4 weeks and 2 weeks respectively prior to acid challenge demonstrated an increase of fluorescence reaching $3.09 \pm 0.51\%$ for Group A and $1.81 \pm 0.52\%$ for Group B.
Figure 7.11

Graph shows the progression of the percentage change of backscattered intensity decay, $\Delta D$, with erosion interval throughout the study period.

At the end of the study period, the groups with natural surface, Group A, B and C, did not demonstrate a distinct change in $\Delta D$. However, in the polished surface group, Group D, marked decrease in $\Delta D$ was observed.
CHAPTER 8

MEASURING EARLY ENAMEL EROSION WITH OCT & QLF \textit{in situ}:

Part II – RANDOMISED CONTROLLED TRIAL OF THE EFFICACY OF A HIGH FLUORIDE DENTIFRICE IN THE PREVENTION OF EARLY EROSION.
8.1. Abstract

Most *in vitro* and *in situ* studies on erosion have used polished enamel samples. Surface enamel is often aprismatic and more highly mineralised than subsurface enamel. Demineralisation studies on natural enamel surface have been carried out showing that polished enamel is more susceptible to erosion than natural enamel. Nonetheless, the remineralisation of natural surfaces has not been investigated.

**Objectives:** The aim of this study was to evaluate the *in situ* effects of two methods of applying a high fluoride dentifrice on the rate of early erosion in human enamel and whether QLF and OCT were able to detect the effects.

**Methods:** It was a single centre, double blind, randomised, 2 product (1 test and 1 control), two treatment phase, split mouth, *in situ* study. 160 teeth were randomly divided into 4 groups (n = 40) : Group A – Natural enamel surface pre-treated for 30 days prior to acid challenge, Group B – Natural enamel surface pre-treated for 15 days prior to acid challenge, Group C – Natural enamel surface with no pre treatment and Group D – Polished enamel with no pre-treatment. The samples in each of these groups were further divided into two sub-groups – Control (C) and Treatment (T) (n = 20 each). The test product used was Duraphat 5000ppm and the control used was deionised water. The study was carried out in two phases. Phase I of the study involved exposing the enamel samples in Group A and B to either the test or control product (2x daily) for 30 and 15 days respectively and immediately followed by Phase II. Phase II of the study involved 5 days of *in vivo* acid challenge (3x/ day, 250mls of orange juice every time) interposed with exposure to the test or control product. All four experimental groups were involved in Phase II of the study. Each of the participants completed a total treatment duration of 35 days. Three dimensional OCT images, QLF images and micro indents with a Knoop micro indenter were made at baseline and at the end of each day of Phase II. Percentage of surface microhardness
change ($\Delta SMC$), decay of backscattered intensity of OCT ($\Delta D$) and percentage loss of fluorescence of the exposed enamel area ($\Delta F$) were analysed.

**Results:** Multiple regression analysis of $\Delta SMC$ with erosion interval of both Treatment and Control sub-groups for all 4 groups showed that surface microhardness detected significant ($P < 0.05$) erosion-interval related decreases. Comparison of the regression coefficients of $\Delta SMC$ with erosion interval for the treatment and control sub-groups of Group C demonstrated significant differences ($P=0.036$) while those of Group D did not demonstrate significant differences ($P=0.248$). Multiple regression analysis of $\Delta F$ with erosion interval for all 4 groups showed significant ($P < 0.05$) erosion-interval related changes of fluorescence. Group C was the only group that showed significantly different regression coefficients between the Treatment and Control sub-groups. However, only $\Delta D$ of Group C and D detected significant ($P < 0.05$) erosion-interval related decrease in the decay of backscattered intensity and $\Delta D$ did not detect any difference between treatment and control sub-groups of all 4 experimental groups.

**Conclusion:** It can be concluded that:

1. Treating natural surface enamel with a 5000ppm NaF dentifrice increased its resistance against early *in vivo* erosion;

2. Pre-treating enamel prior to acid challenge did not increase the resistance of enamel against early *in vivo* erosion and

3. QLF was able to detect the protective effect of a 5000ppm NaF dentifrice on natural surface enamel against early *in vivo* erosion with an orange juice.

4. OCT was not able to detect the protective effect of a 5000ppm NaF dentifrice on natural or polished surface enamel against early *in vivo* erosion with an orange juice.
8.2. Introduction

Prevalence of dental erosion or erosive wear has been reported to be on the rise [Chadwick et al., 2006; Jaeggi and Lussi, 2006], particularly in children [Arnadottir et al., 2010; El Aidi et al., 2010; Kreulen et al., 2010]. Therefore, early diagnosis and adequate prevention are essential to minimize the risk of tooth erosion. Disease prevention includes measures not only to prevent the occurrence of disease such as risk factor reduction, but also to arrest its progress and reduce its consequences once established [WHO, 1984]. Effective prevention of the condition should ideally then be dealt with from both the perspectives of primary and secondary prevention.

8.2.1. Primary Prevention

Primary prevention is directed toward preventing the initial occurrence of a disorder [WHO, 1998]. This can be done in a variety of ways, such as preventing exposures to the causative factor, improving resistance to disease, or education to diminish risk-taking behaviours.

For erosive wear, prevention of exposure to extrinsic acidic sources had been tackled indirectly via the modification of the erosive potential of acidic beverages. Much has been done to modify the erosive potential of acidic beverages by means of modification of pH and manipulation of the ionic content of acidic solutions. The erosive potential of acidic liquid has been found to be highly dependent on its pH, buffering capacity, temperature, presence of chelating agents and the concentration of phosphate, calcium and fluoride [Lussi et al., 1993; West et al., 2000]. The addition of calcium has been shown to reduce the erosive potential of pure acids and acidic drinks, especially on enamel [Barbour et al., 2003; Hughes et al., 2002]. Larsen and Richards [Larsen and Richards, 2002] reported that saturation of acidic beverages with CaF$_2$ reduced the in vitro development of erosion induced by drinks with pH above 3 by 28% but in drinks with pH below 3, erosion was not affected by fluoride concentrations up to 20ppm. Further saturation of the drinks with calcium fluoride did not significantly reduce the
erosive potential of the drinks unless fluoride concentrations in a harmful range were used.

Due to the possibility of a synergistic effect among different ions, the addition of a combination of different ions has also been attempted. Amaechi et al [Amaechi et al., 1998] found that the supplementation of an orange juice with xylitol and fluoride had an additive effect on the reduction of enamel erosion in vitro. Attin et al [Attin et al., 2005] showed that the combination of low level of Ca (0.5 mM), P (0.5mM) and F (0.037mM) is able to reduce enamel loss. Alternatively, efforts have been made to supplement acidic solutions with metal ions such as iron, and later a combination of ions which seems to decrease the erosive potential of acidic solutions [Buzalaf et al., 2006; Kato et al., 2007].

In contrast to the plethora of work that has been carried out to evaluate and improve the efficacy of various fluoride, calcium and phosphate compounds in reducing the rate or erosion, little attempt has been made to evaluate the efficacy of these compounds in improving the resistance of enamel against erosive challenge prior to its occurrence. Hughes et al [Hughes et al., 2004] found that pre-treating enamel with fluoride containing oral hygiene products in vitro offered protection against erosion by citric acid and soft drinks. Lussi et al [Lussi et al., 2008] found better protection of in vitro erosion surface softening with pre-erosion exposure to fluoride and suggested that more research is needed to evaluate this issue.

Primary prevention against erosion and erosive wear can potentially be achieved by enhancing the protective layer or diffusion barrier that is produced following the use of fluoride or other compounds on the tooth surface. Enhancement of the protective layer could either involve increasing the thickness or adherence of the protective layer on the tooth surface.
8.2.2. Secondary Prevention

Secondary prevention seeks to arrest or retard existing disease and its effects through early detection and appropriate treatment [WHO, 1998]. The management strategies for clinically diagnosed erosive wear should involve three stages. The first is assessing and managing identified risk factors, the second is reducing the progress of demineralisation and initiate remineralisation and finally in situations of advanced lesions, restoring function.

8.2.2.1. Management of risk factors

The management strategies are dependent upon the identified factors of an individual and his/her compliance. If the source of acid is extrinsic, management strategies of behavioural factors includes the reduction of the frequency and amount of acid exposure [Eisenburger and Addy, 2003; West et al., 2000], the avoidance of acidic solutions that are of high adherence [Ireland et al., 1995] and modification of the manner of acid exposure that prolongs the duration of an acidic oral environment, such as holding or swishing of acidic beverages in the mouth [Bassiouny and Yang, 2005]. It has also been recommended to avoid tooth brushing immediately after an acidic attack, as it was found that the timing of tooth brushing relative to the erosive challenge significantly influence the amount of erosive wear [Attin et al., 2001; Attin et al., 2004].

Modification of the biological factors mainly revolves around increasing salivary flow. Salivary flow stimulation yields an increase of buffering effect of bicarbonates and mineral content which facilitates calcium and phosphate re-deposition unto the dental hard tissues [Dawes, 1969]. Rios et al [Rios et al., 2006] showed that saliva stimulated by the use of sugar-free chewing gum promoted a remineralising action in the erosive – abrasive situation.

8.2.2.2. Reducing the rate of demineralisation and initiation of remineralisation

The main focus of work so far on the use of oral hygiene product in the management of erosion and erosive wear has been inclined towards their use as a secondary
prevention measure in reducing the rate of ongoing erosion and initiation of the remineralisation of softened enamel [Laurance-Young et al., 2011]. Softened enamel had been remineralised experimentally by exposure to supersaturated solutions \textit{in vitro} [Amaechi and Higham, 2001; Eisenburger et al., 2001] and by exposure to the oral environment \textit{in situ} [Ganss et al., 2007]. Different chemical compounds have been used, such as the topical application of fluoride or calcium-phosphate formulations.

\textit{In vitro} and \textit{in vivo} studies have emphasised the importance of maintaining a concentration of fluoride (F) in oral fluids that is effective in reducing the rate of demineralisation. Having these ‘bio-available’ F reservoirs in the vicinity of the site of demineralisation and remineralisation persistently increased the concentration of F in the oral fluids [Marinho, 2008]. One of the primary sources of F reservoir in the oral environment is the mineral deposits of F as calcium fluoride (CaF$_2$) [Larsen and Ravnholt, 1994; Ogaard, 2001]. CaF$_2$ – like deposits can be formed by the reaction of tooth-bound calcium (Ca) with the applied F.

\textbf{8.2.2.2.1. Sodium Fluoride and Amine Fluoride}

The main mechanism of action of conventional fluoride compounds such as sodium fluoride (NaF) and amine fluoride (AmF) in preventing demineralisation is mainly related to the formation of calcium fluoride (CaF$_2$) layer [Ganss et al., 2007; Saxegaard and Rolla, 1988]. The protective role of CaF$_2$ is thought to be two pronged. Firstly it poses a physical barrier against direct contact of the underlying surface with acid and secondly acts as a mineral reservoir for remineralisation [Magalhaes et al., 2011].

The formation of the CaF$_2$ – like deposits and its protective effect against demineralisation is highly dependent on the pH, the concentration of fluoride and its frequency of application. The deposition of CaF$_2$ on the surface increases with increasing concentration and frequency of application and decreasing pH of the agent. Fluoride agents with a pH < 5 seem to induce a higher CaF$_2$ deposition on a dental surface than pH neutral ones [Saxegaard and Rolla, 1988].
Ganss et al [Ganss et al., 2001] used longitudinal microradiography to assess cumulative in vitro mineral loss over 5 days. They found that intensive fluoridation significantly reduced erosion progression in enamel and had a even more pronounced effect on dentine. The samples were immersed in citric acid 6x10 min/day and subjected to 2 different fluoridation regimes. One was by dentifrice fluoridation only (NaF, 0.15% F-) and the other was dentifrice fluoridation with the additional application of a fluoride mouth rinse (SnF2, 0.025%F-) 3x5 and gel fluoridation (NaF; 1.25% F-).

After the first experimental day, no significant differences were found between the groups. However, after 5 days the erosive mineral loss values between the two groups were significantly different. As the anti-erosive effect of conventional fluorides requires a very intensive fluoridation regime, more recent studies have focused on fluoride compounds that contain polyvalent metal ions such as stannous fluoride and titanium tetrafluoride.

8.2.2.2.2. Stannous Fluoride

Babcock et al [Babcock et al., 1978] attributed the mechanism of demineralisation protection of tin-containing fluoride compound to the formation of metal rich precipitates - [Ca(SnF3)2, SnOHPO4, Sn3F3PO4], which had been shown to be highly resistant to acid. In addition to that, tin was also found to penetrate and become incorporated into demineralised enamel when high concentration tin containing fluoride mouth rinses were used [Schlueter et al., 2009].

Ganss et al [Ganss et al., 2008] evaluated the relevance of cations in different fluoride compounds for their effectiveness as anti-erosive agents and showed that SnCl2 (800 ppm tin), NaF (250 ppm fluoride), AmF/SnF2 (250 ppm fluoride/390 ppm tin) and SnF2 (250 ppm fluoride/809 ppm tin) solutions could reduce enamel erosion and solutions containing SnF2 were most effective.

Acidic solutions, such as native solutions of stannous fluoride (SnF2), titanium tetrafluoride (TiF4) and hydrofluoric acid (HF), have been shown to be effective in vitro
and *in situ* [Hjortsjo et al., 2010; Hove et al., 2008]. Of the above, SnF$_2$ has recently been considered the most promising, as both TiF$_4$ and HF are probably too acidic for clinical use (pH < 2), and pH adjustment of TiF$_4$ reduces its protective effect [Wiegand et al., 2009]. SnF$_2$ is already being used in dentifrices and mouth rinses, and its effect on plaque and gingivitis is well recognized [Paraskevas and van der Weijden, 2006].

### 8.2.2.2.3. Titanium tetrafluoride, TiF$_4$

Several *in vitro* studies have reported an inhibitory effect of 0.4 – 10% TiF$_4$ solution on dental erosion [Hove et al., 2007; van Rijkom et al., 2003]. This protective effect has been attributed not only to fluoride but also to the formation and incorporation of a new compound, hydrated hydrogen titanium phosphate, into the hydroxyapatite lattice [Buyukyilmaz et al., 1997].

Proof of efficacy of TiF$_4$ under clinical conditions is scarce and contradictory. Whilst only two *in situ* studies showed 1.6% TiF$_4$ (0.5 m fluoride) to be as effective as SnF$_2$ or AmF in the prevention of erosion [Hove et al., 2008; Wiegand et al., 2010], others did not show any protective effect of 4% TiF$_4$ [Magalhaes et al., 2008; Vieira et al., 2005].

### 8.2.2.2.4. Calcium Phosphate

The binding of Ca and F is pivotal in the formation of bioavailable oral F reservoirs. However, salivary free-Ca is typically only 2 – 10% of the amount of F supplied by topical agents [Vogel, 2011]. Thus, the amount of oral Ca-F that can be formed after use of a topical F agent is limited not only by the concentration of applied F, but also by the rate at which additional Ca can be found. Casein phosphopeptides (CPP) have the ability to stabilise amorphous calcium phosphates (ACP) in metastable solution and have demonstrated their preventive properties in the caries process [Cochrane et al., 2010]. The evidence for its effect on erosion however is contradictory. Ranjitkar et al [Ranjitkar et al., 2009] compared the efficacy of a CPP-ACP product against a negative control in the reduction of erosive wear in enamel and dentine *in vitro*. They found that the CPP-ACP product significantly reduced enamel and dentine erosive and abrasive
wear. However, Wegehaupt et al [Wegehaupt et al., 2011] in a recent in situ study found that intra-oral application of a CPP-ACP crème provided no benefit regarding re-hardening of enamel softened by erosion.

8.2.2.3. Modes of application
The above compounds can be delivered to the tooth surface by different means. The most common mode of delivery is the dentifrice. Other modes of delivery used are in the form of mouth rinses, gels and varnishes.

In an in situ study, Zero et al [Zero et al., 2006] found a protective dose response of fluoride in dentifrice after 25 minutes of in vitro exposure to grapefruit juice. The two dentifrices with fluoride contents of 1100ppm and 1150 ppm significantly enhanced remineralisation of softened enamel when compared to a placebo dentifrice. In another study [Lussi et al., 2008], the impact of 5 different dentifrices on enamel demineralisation and remineralisation was investigated in vitro. Erosion was produced by with immersion in citric acid (1%, pH 4) for 3 min. No statistically significant differences were found among the dentifrices.

Different groups of researchers have reported increased resistance against severe erosive wear after the application of a highly fluoridated gel with a pH of 4.75 [Attin et al., 1999; Ganss et al., 2001]. Ganss et al [Ganss et al., 2001] suggested that after application of the gel, some mineral dissolved from the enamel surface, increasing the local pH and leading to precipitation of CaF$_2$ – like material, which leads to less softening than without this layer.

Fluoridated varnishes were introduced into the market in the 1960s and were intended for professional application only. The main advantages of varnishes are the prolonged contact time between fluoride and the tooth surfaces, hence it increases fluoride uptake by dental hard tissues as well as the formation of CaF$_2$ reservoirs. These products contains higher concentration of fluoride compared to gels with typical concentrations
of 22,600 ppm in NaF varnishes, 7,000 ppm fluoride (in difluorosilane varnishes) or 56,300 fluoride in 6% NaF + 6 % CaF2 varnishes [Marinho et al., 2002].

In recent years, research groups have investigated the preventive effect of several fluoride regimes on dental erosion and found differing efficacy [Attin et al., 1999; Ganss et al., 2001; Ganss et al., 2011; Hughes et al., 2004; Lussi et al., 2008; Schlueter et al., 2009; van Rijkom et al., 2003]. Comparison of efficacy of the various F formulations is difficult because the protocols, details and parameters of the interventions studied varied tremendously.

Larsen and Richards [Larsen and Richards, 2002] showed in vitro that fluoride treatment was unlikely to provide a preventive effect against erosion because an acidic drink will rapidly dissolve accessible calcium fluoride and remove the remaining traces of a previous topical fluoride treatment. However, it has to be kept in mind that the calculations and measurements of this study did not take into account the coating of the CaF2 layer by phosphates and proteins, which occurs in the mouth. It has been found that these in vivo or in situ stabilized CaF2 particles are more resistant to an acidic attack than ones produced in vitro [Ganss et al., 2007; Ogaard et al., 1994]. In one study, fluoride dentifrice when compared to a similarly formulated dentifrice except for fluoride content, significantly reduced the wear of eroded enamel to brushing abrasion when immersed in vitro in a cola drink for 5 min, 4 times a day [Magalhaes et al., 2007]. Bartlett et al. [Bartlett et al., 1994], using a cycling model of erosion (citric acid, 5 min, pH 3.5) and abrasion, showed less wear in the presence of fluoride dentifrice than in the presence of a non-fluoride dentifrice.

Lippert et al [Lippert et al., 2004] focused on the potential process of enamel remineralisation by particularly paying attention to the possible re-hardening of surface softened enamel and employing physiologically relevant substrates, demineralisation/remineralisation times and solutions and did not find enamel re-hardening. This is in contradiction to previous studies [Feagin et al., 1969; Wei and
Koulourides, 1972], which reported a re-hardening of enamel but these previous studies employed remineralising solutions with higher calcium and phosphate concentrations and a molar calcium/phosphate ratio close to that of a stoichiometric hydroxyapatite.

Most of the above studies mainly concentrated on the investigation of the remineralisation of carious lesions and eroded enamel. However, not much attention has been paid to increasing the resistance of sound natural enamel from erosion. Lippert et al [Lippert et al., 2004] has suggested that more research is necessary to fully understand the processes involved in the early stages of enamel demineralisation and remineralisation as they are of high physiological importance.
8.3. Objectives and Working Hypothesis

The aim of this part of the study was to evaluate with surface microhardness, QLF and OCT the \textit{in situ} effects of two methods of applying a high-concentration fluoride dentifrice on the rate of early erosion in human enamel.

The objectives of the study were:

1. To evaluate whether there was a protective effect of pre-treating human enamel with a high-concentrated fluoride dentifrice prior to the onset of acid challenge.
2. To evaluate whether there was a protective effect of treating human enamel with a high-concentrated fluoride dentifrice during an active erosion phase.
3. To evaluate whether there is a difference in the efficacy of a high-concentrated fluoride dentifrice in reducing the rate of early erosion between polished and natural surface human enamel.

The Null Hypotheses tested were:

1. Pre-treating natural surface enamel with a high fluoride dentifrice regime does not increase the resistance of enamel against \textit{in vivo} erosive challenge regime with an orange juice.
2. Treating natural surface enamel with a high fluoride dentifrice does not increase its resistance against concurrent early \textit{in vivo} erosion with an orange juice challenge regime.
3. Treating polished-surface enamel with a high fluoride dentifrice does not increase its resistance against early \textit{in vivo} erosion with an orange juice challenge regime.
4. There is no difference in the effect of treating natural surface or polished surface enamel with a high fluoride dentifrice.
5. QLF is not able to detect the protective effect of a high fluoride dentifrice on natural or polished surface enamel against early \textit{in vivo} erosion with an orange juice challenge regime.
6. OCT is not able to detect the protective effect of a high fluoride dentifrice on natural or polished surface enamel against early \textit{in vivo} erosion with an orange juice challenge regime.
8.4. Materials and Methods

8.4.1. Study design

This was a part of the study described in Chapter 7. It was a single centre, double blind (blinded to the research participants and the persons responsible for performing the sample analysis), randomised, 2 product (1 test and 1 control), two treatment phase, split mouth, in situ study. The test product used was Duraphat 5000ppm (Colgate-Palmolive, Guildford, United Kingdom) and the control used was deionised water.

8.4.2. Enamel samples

160 intact, caries-free human incisor teeth, that were recently extracted from patients aged 18 or over, of either gender, were collected following ethical approval. All teeth were soaked in 20,000 ppm sodium hypochlorite to water solution for at least 24 hours, as a disinfection procedure [Kohn et al., 2003] and then scraped clean of any remaining tissue with a scalpel. The teeth were then washed in distilled water. The teeth were randomly divided into 4 groups (n = 40) as described below.

Group A – Natural enamel surface pre-treated for 30 days with the test product or control prior to acid challenge

Group B – Natural enamel surface pre-treated for 15 days with the test product or control prior to acid challenge.

Group C – Natural enamel surface with no pre-treatment prior to acid challenge

Group D – Polished enamel surface with no pre-treatment prior to acid challenge

The samples in the above groups were divided further into two sub-groups – Control (C) and Treatment (T) (n = 20 each). Figure 8.1 presents the division of the enamel samples in the 4 main groups and their corresponding Treatment and Control sub-groups.
The preparation of the natural surface enamel samples, the polished surface enamel samples and the non-exposed areas of these samples were as described in Sections 7.4.2.1, 7.4.2.2 and 7.4.2.3.

8.4.3. Recruitment of participants

The recruitment of participants was as described in Section 7.4.3.

8.4.4. Intra-oral appliances

Right and left mandibular buccal customised intra-oral appliances were made for each participant and randomisation was performed to determine which product was applied to the right and left appliances. The acrylic appliances were colour-coded distinguish the right-side ones from the left-side ones. A lower alginate impression was recorded in a perforated stock tray and then poured in Kaffir D dental stone within 30 minutes and lower left and right intra-oral appliances was constructed. Cribs were constructed to fit suitable posterior teeth to aid retention. A channel was made along the buccal aspect of each appliance to accommodate one enamel sample from each experimental group. Three out of these four enamel samples were samples with untouched, natural surface samples while the fourth one had a polished surface. The positions of the enamel samples of the different groups in the appliance were randomly allocated.

8.4.5. In situ phase of study

The study was carried out in two phases. Phase I of the study involved exposing the enamel samples in Group A and B to either the test or control product for different periods of time and immediately followed by Phase II. Phase II of the study involved 5 days of in vivo acid challenge interposed by exposure to the test or control product. All four experimental groups were involved in Phase II of the study. Each of the participants completed a total treatment duration of 35 days (Figure 8.1).
8.4.5.1. Phase I - Exposure of enamel samples in Group A and B to fluoride

For the first 15 days of treatment, each appliance was fitted with one enamel sample of Group A and 3 ‘blank’ samples (A blank sample is a block of resin the same size and shape as the enamel samples). The enamel samples were placed randomly at any of the four sample slots of the intra-oral appliance as assigned by a random table generated using SPSS v 16.0.

The subject wore the appliances between 9:00 +/- 30 minutes and 16:30 +/- 30 minutes. The appliances were removed from the mouth for up to a 1 hour period over lunch. Each subject wore the appliances a minimum of 6 hours daily. Subjects attended the site twice a day for ex vivo treatment application of the test and control product at 09:00 +/- 30 minutes and 15:30 +/- 30 minutes.

The dentifrice was applied by placing the appliances fitted with the enamel samples of the treatment sub-group into a small glass beaker containing a slurry of the dentifrice. This slurry was made from 9g of paste to 30ml of distilled water (pH = 8.15 ± 0.2). The appliance fitted with the enamel samples of the control sub-group was placed in 30ml of deionised water. The beakers were then placed on an orbital shaker at a resolution of 120rpm for 2 minutes. Thereafter, the appliances were removed from the slurry and replaced into the subjects’ mouth until the next treatment. A fresh preparation of the slurry and fresh supply of deionised water were used for each treatment application.

The appliances were returned to the study site at 16:30 ± 30 minutes each day and were stored in a ‘moist pot’ overnight (The pot contained a damp cotton wool pad, moistened with water). Similarly, at those times during treatment days when it is necessary to take the appliances out of the mouth, for reasons other than to receive treatment, they were placed into a ‘moist pot’ to prevent the enamel samples drying out. Participants were issued a standard dentifrice (Colgate® Cavity Protection) and toothbrush (Colgate® Total® Professional) to use at home for the whole duration of the study.
At the start and end of each study day, the intra-oral appliances were disinfected with 70% ethanol spray, left for 2 minutes and then rinsed under running water. A soft bristle toothbrush was used to remove any debris and plaque formation on the appliances and samples.

On Day 16, one sample from Group B was added to the appliance randomly as assigned by a random table. This daily protocol was then repeated for an additional 15 days. At the end of Day 30, following disinfection, the enamel samples were removed from the appliances and baseline measurement taken using surface microhardness, quantitative light fluorescence (QLF) and optical coherence tomography (OCT). After measurements were taken, the enamel samples were placed back into the intra-oral appliances and kept in the moist pots as described above.

8.4.5.2. Phase II - Acid challenge of all study groups

On Day 31, one sample each from Group C and Group D was added to the remaining two empty two slots, as assigned by the generated random table mentioned above. During this phase of study, as per Phase I of the study, the participants continued to wear the right and left intra-oral appliances from 9:00 +/- 30 minutes to 16:30 +/- 30 minutes except for a 1 hour period over lunch, together with the ex vivo treatment regime at 09:00 +/- 30 minutes and 15:30 +/- 30 minutes.

In addition to the above, there were three in vivo acid challenges daily. Immediately after the first treatment application (09:00 +/- 30 minutes), each subject will return the appliance to the mouth and swish with a total of 250ml of orange juice over a 10 minute period, under supervision of a member of the study site staff. A second acid challenge took place at 13:00 +/- 30 minutes and a third, immediately prior to the treatment application, at 15:30 +/- 30 minutes.

During these in vivo acid challenges, the participants were asked to orally swish with 25ml of orange juice, Sainsbury’s Smooth Orange Juice from Concentrate (Sainsbury’s
Supermarkets Ltd, 33 Holborn, London EC1N 2HT) (pH = 3.4 ± 0.1), for 1 minute, expectorate, and then swished with a fresh 25ml of orange juice again. This was repeated until participants had swished with 25ml of orange juice 10 consecutive times, thereby exposing the enamel samples to 250 ml of the acidic beverage over a 10 minute period. A total of 750ml were swished around the mouth for a total of 30 minutes each day, on alternate days, over a 2 week period.

8.4.6. Measurement of samples

The appliances were returned to the study site at least 1 hour after the last acid challenge of each day. Prior to performing any measurements on the enamel samples, the appliance were sprayed with a 70% ethanol solution, left for 2 minutes and then rinsed under running water. Instead of using a soft bristle toothbrush to remove any debris and plaque as was done during Phase I of the study, the surface of the enamel sample were wiped with 0.2% Chlorhexidine Gluconate [Azzopardi et al., 2004] using a micro brush. This was necessary to avoid tampering with the softened eroded surfaces.

Following disinfection, the enamel samples were removed from the appliance and placed in humidified Eppendorf receptacles prior to being measured using surface microhardness, quantitative light fluorescence (QLF) and optical coherence tomography (OCT). The protocols of measurements with these instruments were as described in Sections 7.4.6.1, 7.4.6.2, and 7.4.6.3.

The outcome measure for surface microhardness was expressed as the percentage of surface microhardness change ($\Delta SMC$), calculated based on the differences between Knoop hardness numbers at baseline, $KHN(t_0)$ and the subsequent erosion intervals, $KHN(t)$. $\Delta SMC$ was calculated as:

$$\Delta SMC(t) = 100 \left( \frac{KHN(t) - KHN(t_0)}{KHN(t_0)} \right)$$
The outcome measure for QLF was expressed as the percentage loss of fluorescence of the exposed area at each erosion interval, $\Delta F(t)$, and was calculated as follows:

$$\Delta F(t) = 100 \left[ \frac{F_E(t)}{F_E(t_0)} - \frac{F_{NE}(t)}{F_{NE}(t_0)} \right]$$

where $t_0$ is the baseline time point.

The outcome measure for OCT was the percentage of change in decay of backscattered intensity of the exposed area at a particular erosion interval, $\Delta D(t)$, compared to baseline, $D_E(t_0)$. $\Delta D(t)$ for each measurement time point is represented by the function shown below:

$$\Delta D(t) = 100 \left[ \frac{D_E(t) - D_E(t_0)}{D_E(t_0)} \right]$$

where $D_E$ is $D$ of the exposed area, $t_0$ is the baseline time point and $t$ is the specific measurement time point. Again, the decay of the backscattered light, $D$, is the relationship of the attenuation of backscattered light between two optical depths of an OCT A-Scan and is represented by the function below,

$$D(t) = \frac{I_{\text{plateau}}(t)}{I_{\text{superficial}}(t)}$$

$I_{\text{superficial}}$ is the intensity of backscattered light at or immediately below the enamel-air interface where demineralisation had occurred and $I_{\text{plateau}}$ is the backscattered intensity where the A-scan had reached a plateau and assumed not to be affected by the erosive challenge (Figure 5.3). As in the previous studies described in Chapter 5, 6 and 7, the chosen superficial optical depth for this study was 30µm below the tooth air interface and the level of plateau was observed to be 150 µm below tooth-air interface.
8.4.7. Statistical Analysis

The STATA™ 10.1 (Statacorp, Texas) statistical program was used. Multiple linear regression analyses were performed, taking into account the clustering of samples using robust variance estimates, to ascertain whether erosion-interval (time) related changes were detected in both the control and treatment groups for all the four experimental groups.

When a significant time-related change was found for an outcome measure for the control and treatment group, comparison of the regression coefficient was undertaken to determine whether the rate of time related changes were different between the control and treatment groups.
8.5. Results

8.5.1. Surface microhardness

8.5.1.1. Natural surface samples

Multiple regression analysis of $\Delta SM_C$ with erosion interval of both Treatment and Control sub-groups for all the three natural surface groups showed that surface microhardness detected significant ($P < 0.05$) erosion-interval related decrease. Hence erosion-interval is a significant co-variate for the decrease of surface microhardness. Table 8.1 presents the results of the analysis.

Figure 8.2 is the line graph that shows progression of $\Delta SM_C$ for the Treatment sub-groups of all the four experimental groups. At the end of the study period, the mean cumulative percentage decrease of surface microhardness observed for Group A (exposed to fluoride for 4 weeks prior to acid challenge) was 30.33 ± 5.11% (mean ± S.E.), for Group B (exposed to fluoride for 2 weeks prior to acid challenge) - 39.78 ± 3.66% and for Group C (no prior exposure to fluoride) – 27.00 ± 5.28%. The regression coefficients of these three Treatment sub-groups were compared to assess whether the slope of the erosion-interval related decrease were significantly different. Although Group C showed less decrease of surface microhardness compared to the other two groups of natural surface specimens, it was found that the regression coefficients of these three groups were not significantly different ($P > 0.05$) from each other.

Figure 8.3 shows line graphs comparing the mean decrease of surface microhardness between treatment and control sub-groups of each experimental group. Although initially the Control sub-group in Group A displayed less decrease in surface microhardness, the Control sub-groups of all three experimental groups demonstrated more decrease in surface microhardness than their respective Treatment sub-groups at the end of the study. The difference (between the two sub-groups) in decrease of
surface microhardness at the end of the study for Group A was 2.37%, for Group B was 10.80% and for Group C was 26.97%.

Comparison of the regression coefficient between the treatment and control sub-groups of each of the three experimental groups was undertaken. It was found that only the treatment and control sub-groups of Group C demonstrated significant difference (P=0.036) in erosion-interval related decrease of surface microhardness (Table 8.4).

8.5.1.2. Polished surface samples

Multiple regression analysis of $\Delta SMC$ with erosion interval of both Treatment and Control sub-groups for Group D showed that surface microhardness detected significant (P < 0.05) erosion-interval related decrease. Hence erosion-interval is a significant co-variate for the decrease of surface microhardness (Table 8.1).

The line graph in Figure 8.3 shows the progression of $\Delta SMC$ of both the sub-groups. At the end of the study period, the mean cumulative percentage decrease of surface microhardness observed for the Control sub-group was 81.05 ± 2.66 % (mean ± S.E.) and 76.6 ± 2.83 % for the Treatment sub-group. The regression coefficients of these two sub-groups were compared and although the Treatment sub-group showed less decrease of surface microhardness compared to Control sub-group, it was found that the regression coefficients of these two sub-groups were not significantly different (P > 0.05).

8.5.2. QLF

8.5.2.1. Natural surface samples

Multiple regression analysis of $\Delta F$ with erosion interval for all these three groups and their sub-groups showed that QLF detected significant (P < 0.05) erosion-interval related change of fluorescence. Table 8.2 presents the results of the analysis. Both the treatment and control sub-groups of Group A and B demonstrated positive
relationships with erosion interval while those of Group C demonstrated a negative relationship with erosion interval.

Figure 8.4 are line graphs comparing the mean loss of fluorescence between treatment and control sub-groups of each experimental group. Throughout the study period, both the treatment and control sub-groups of Group C demonstrated a loss of fluorescence. The cumulative loss of fluorescence for the Control sub-group was more than the Treatment sub-group, with -2.83 ± 0.71 (Mean ± S.E.) on Day 1 and reached -4.74 ± 0.58 % on Day 5, while the loss of fluorescence of the Treatment sub-group was recorded at -1.76 ± 0.61 at Day 1 and reached -3.2 ± 0.72 at Day 5. However, Group A and B which were exposed to the test or control product for 30 days and 15 days respectively prior to acid challenge, demonstrated an increase of fluorescence throughout Phase II.

The regression coefficients of the Treatment and Control sub-groups of Group A and B were compared and it was found that the slope of the erosion-interval related increase in fluorescence between the sub-groups in Group A and B did not differ significantly (P > 0.05). Comparison of the regression coefficients between the two sub-groups of Group C, however differed significantly (P= 0.042).

**8.5.2.2. Polished surface samples**

Multiple regression analysis of $ΔF$ with erosion interval of the Treatment and Control sub-groups of Group D showed that QLF detected significant (P < 0.05) erosion-interval related loss of fluorescence (Table 8.2).

As shown in Figure 8.4, Group D showed a similar trend of a loss of fluorescence to Group C throughout Phase II of the study, though to a lesser extent. The cumulative loss of fluorescence for the Control sub-group was more than the Treatment sub-group reached -1.51 ± 0.58 % on Day 5, while the loss of fluorescence of the Treatment sub-group reached -0.48 ± 0.36 at Day 5.
However, comparison of the regression coefficients between the two sub-groups of Group D did not differ significantly (P>0.05).

8.5.3. OCT

8.5.3.1. Natural surface samples

Multiple regression analysis of $\Delta D$ with erosion interval for all these three groups and their sub-groups showed that only the treatment and control sub-groups of Group C showed significant erosion-interval related decrease in the decay of backscattered intensity (Treatment sub-group, $P < 0.0001$; Control sub-group, $P=0.010$). Both the treatment and control sub-groups of Group A and B did not detect significant ($P > 0.05$) erosion-interval related changes in the decay of backscattered intensity. Table 8.3 presents the results of the analysis.

Figure 8.5 are line graphs comparing the mean decay of backscattered intensity, $\Delta D$ between treatment and control sub-groups of each experimental group. Throughout the study period, both the Treatment and Control sub-groups of Group C demonstrated a decrease in the decay of backscattered intensity. The cumulative decrease in decay of backscattered intensity for both the Control and Treatment sub-group reached a maximum of about 10 % (Control sub-group – $10.56 \pm 3.51$%; Treatment sub-group – $13.43 \pm 5.26$) by Day 3 of the study. Comparison of the regression coefficients between the two sub-groups of Group C however did not differ significantly ($P= 0.583$).

8.5.3.2. Polished surface samples

Multiple regression analysis of $\Delta D$ with erosion interval of the Treatment and Control sub-groups of Group D showed that OCT detected significant ($P < 0.05$) erosion-interval related decreases of backscattered intensity decay in Group D, as shown in Table 8.3.
The line graph of Group D in Figure 8.5 showed the decay of backscattered intensity decreased markedly during the study period. For the Control sub-group, there was a mean $\Delta D$ of 34.71 ± 5.63 by Day 3 of erosive challenge, which reached 40.57 ± 5.58 % at the end of the study. The progression of $\Delta D$ in the Treatment sub-group was observed to be similar to that of the Control sub-group, though more gradual. The cumulative decrease in $\Delta D$ observed at the end of the study period for the Treatment sub-group was 42.70 ± 5.40 %. Comparison of the regression coefficients between the two sub-groups of Group D showed that the decrease in decay shown by these two sub-groups did not differ significantly (P= 0.88).
8.6. Discussion

8.6.1. Study Design

Most conclusions of previous studies on the efficacy of preventive products for erosion were based upon studies that had used polished enamel as a substrate. This was mainly because the assessment tools used, such as surface profilometry require polished samples. Nevertheless, it could be debated that using polished samples bear some clinical relevance as the ultrastructure of a polished sample could be similar to a clinically active eroding surface [Huysmans et al., 2011].

However, it is of equal clinical significance to attempt to use samples with natural surfaces as the two types of surfaces are ultrastructurally different [Meurman and Frank, 1991]. Little research has been conducted to discern the relative susceptibility of natural and polished surfaces to erosive challenge and responses to preventive therapies. Ganss et al [Ganss et al., 2000] in one of the few studies that have attempted to look into the above issue found that in an in vitro advanced erosion model, natural surfaces showed significantly smaller erosion depths than polished samples. Nevertheless, no studies to date had attempted to compare the efficacy of preventive agents between these two substrates, hence the inclusion of polished enamel surface samples in this study.

The objective of pre-treating the natural surface enamel samples with a fluoridated dentifrice was to simulate a primary prevention model in an attempt to increase the resistance of enamel against the occurrence of erosion and erosive wear. Having two pre-treatment groups of different pre-treatment intervals would potentially provide insights into whether increasing the length of pre-treatment will also increase the level of protection. Previous in vitro work had been carried out by different groups of researchers into the effects of pre-treating enamel samples with fluoride prior to acid challenge. Lussi et al [Lussi et al., 2008] studied two different protocol of application of 5 different fluoridated dentifrices (4 NaF formulation with different pH and 1 SnF$_2$
formulation): (1) Incubation in dentifrice slurry followed by acid softening and artificial saliva exposure; (2) Acid softening followed by incubation in dentifrice slurry and artificial saliva exposure. They found that the incubation of enamel specimens in dentifrice slurry prior to softening seems to be more favourable than post exposure incubation. The authors speculated that the observation could be due to the incorporation and / or deposition of CaF$_2$-like material on to the enamel surface which led to less softening than in the absence of this layer [Ganss et al., 2001; Lussi and Hellwig, 2001]. The same was also discussed in two other previous studies which had also looked into pre-treating samples with fluoride before they are subjected to erosive challenge [Hughes et al., 2004; Ponduri et al., 2005].

### 8.6.2. Surface microhardness results

The Treatment sub-groups of Group A, B and C (natural surface) all showed decrease in surface microhardness as the erosive challenge progressed. This meant that neither pre-treating enamel samples with concurrent fluoride treatment nor just concurrent fluoride treatment halted the erosion process. Comparison between the Treatment and Control sub-groups within each experimental group revealed that only Group C showed a significant difference between the two sub-groups. Although no significant differences were found in the corresponding Treatment and Control sub-group of Group A and B, the separation was greater in Group B than Group A (Figure 8.3). Whilst samples of the Treatment sub-group were being treated with fluoride during Phase I of the study, the Control sub-group were immersed in distilled water and both groups were exposed to saliva for 30 or 15 days.

It seems that exposure of the Control sub-group of Group A to the oral environment for 30 days had afforded the samples a similar level of protection as fluoride treatment for the Treatment sub-group for the same length of time. In Group B however, the Control sub-group exhibited more cumulative decrease in surface microhardness (albeit not statistically significant) than the corresponding Treatment sub-group and it could be
speculated that the shorter exposure time to the oral environment resulted in a smaller bio available F reservoir. The result in Groups A and B is possibly different from Lussi et al’s [Lussi et al., 2008] results because in his study, after pre-treating the samples and after erosion, there was no subsequent treatment of samples as there was in Phase II of the present study. So the protective effect of the pre-treatment that might have occurred during Phase I of this present study could have been masked by the concurrent treatment of the samples during the erosive challenge period in Phase II.

As shown in this study and that of Ganss et al [Ganss et al., 2000], the rate of erosion of polished surface samples was far greater than that for natural surface samples. Lippert et al [Lippert et al., 2004] has shown that a surface deposit, which consisted of a fairly rough crystalline phase (Fig. 3c of that paper), did not protect the underlying enamel from a subsequent acid attack (Fig. 1 of that paper). Furthermore, this surface layer was completely removed even by a short exposure to an erosive solution (Fig. 3d of that paper). The obtained surface softening of the enamel samples after the exposure to a remineralisation solution (Fig. 1 of that paper) can be explained by the fact that the deposited surface material with its loosely packed crystals influenced the nanoindentation measurements to a great extent.

Hence the amount of CaF$_2$-like molecules deposited might not have been sufficient to create a F reservoir that could tip the de- and remineralisation balance towards the latter. The results of no difference in the change of surface microhardness between the treated and non treated sub-groups of the polished surface samples concur with the findings of some work [Ganss et al., 2007] which looked into the efficacy of neutral pH fluoridated dentifrice in the prevention of erosion.

However, the decrease in surface microhardness of Treatment and Control sub-group of Group C differ significantly. This meant that treating natural surface enamel with high concentrated fluoride dentifrice concurrently with active erosion did reduce surface softening. It would be clinically relevant to repeat some of the previous studies on the
efficacy of the various fluoride compounds on erosion using natural surface enamel samples. Negative results of previous studies on polished enamel samples may not necessarily be replicated on natural surface enamel samples.

8.6.3. QLF results

A significant net increase of fluorescence (over the study period) of the exposed area was observed in all the Treatment and Control sub-groups of Group A and Group B (Figure 8.4). The net increase in fluorescence observed in both sub-groups of Group A were more than those of Group B, may, as speculated in Chapter 7 be the result of stain uptake of the non-exposed area during the two different periods of exposure to the oral environment. If true, this would explain why QLF data for these two groups could not show trends of demineralisation and/or remineralisation of the exposed area. Future work involving the use of QLF in in situ studies to longitudinally monitor early erosion may have to have either have in place strict anti-staining protocols or involve relatively shorter study periods.

As for the two experimental groups that were not subjected to pre-treatment, significant loss of fluorescence was detected as the erosion challenge progressed. Nevertheless, in contrast to the surface microhardness results, the rate of loss of fluorescence in Group C was more than that observed in Group D in both the Treatment and Control sub-groups. This could be because the polished enamel surface is more porous than the natural surface enamel sample and therefore picking up fluorescent material from the mouth and/or the dentifrice more readily than a natural surface.

The trend of loss of fluorescence throughout the study period in the Treatment and Control sub-group of Group C mirrors the surface microhardness results of the same experimental groups. Significant difference in loss of fluorescence was also only detected between the sub-groups of Group C but not for Group D. QLF could be used to longitudinally monitor early erosive and remineralisation changes under the current
study conditions for natural surface samples which were not pre-treated in the oral environment.

8.6.4. OCT results

Erosion-interval related decay of backscattered intensity was detected in Group C and D while this was not the case for the two pre-treatment groups (A and B). This phenomenon could be related to the masking of the backscattered intensity by the presence of mature pellicle on the exposed area in Group A and B.

The significant difference demonstrated between the Treatment and Control sub-groups of Group C by surface microhardness and QLF was not detected by OCT. More in depth studies are needed to look into the effects of mature pellicle and plaque on OCT signals and ways to overcome this problem.
8.7. Conclusions

1. Pre-treating natural surface enamel with a 5000ppm NaF dentifrice did not increase its resistance against early *in vivo* erosion with an orange juice challenge regime.

2. Treating natural surface enamel with a 5000ppm NaF dentifrice increased its resistance against concurrent early *in vivo* erosive challenge with an orange juice challenge regime.

3. Treating polished surface enamel with a 5000ppm NaF dentifrice did not increase its resistance against concurrent early *in vivo* erosive challenge with an orange juice challenge regime.

4. QLF was able to detect the protective effect of a 5000ppm NaF dentifrice on natural surface enamel against early *in vivo* erosion with an orange juice challenge regime.

5. OCT was not able to detect the protective effect of a 5000ppm NaF dentifrice on natural or polished surface enamel against early *in vivo* erosion with an orange juice.
8.8. References


WHO: Glossary of Terms Used in Health for All. 1984.


8.9. Tables

Table 8.1

Multiple regression analysis of $\Delta SMC$ with erosion interval for the Treatment and Control sub-groups of the 4 experimental groups.

<table>
<thead>
<tr>
<th>Group</th>
<th>Control P value</th>
<th>F Treatment</th>
<th>Coefficient Control</th>
<th>F Treatment</th>
<th>Control $R^2$</th>
<th>F Treatment $R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>&lt; 0.000</td>
<td>&lt; 0.000</td>
<td>-0.060</td>
<td>-0.049</td>
<td>0.206</td>
<td>0.115</td>
</tr>
<tr>
<td>B</td>
<td>&lt; 0.000</td>
<td>&lt; 0.000</td>
<td>-0.083</td>
<td>-0.065</td>
<td>0.346</td>
<td>0.342</td>
</tr>
<tr>
<td>C</td>
<td>&lt; 0.000</td>
<td>&lt; 0.000</td>
<td>-0.078</td>
<td>-0.050</td>
<td>0.184</td>
<td>0.143</td>
</tr>
<tr>
<td>D</td>
<td>&lt; 0.000</td>
<td>&lt; 0.000</td>
<td>-0.139</td>
<td>-0.132</td>
<td>0.594</td>
<td>0.533</td>
</tr>
</tbody>
</table>

$\Delta SMC$ of all four experimental groups detected significant ($P < 0.05$) erosion-interval related decrease (coefficients with negative value). Hence erosion-interval is a significant co-variate for the decrease of surface microhardness in all the treatment and control sub-groups of the 4 experimental groups. The control and treatment sub-group of Group D demonstrated the fastest decrease (coefficient of -0.139 and -0.132 respectively) when compared to the other 3 experimental groups.
Table 8.2

Multiple regression analysis of $\Delta F$ with erosion interval for the Treatment and Control sub-groups of the 4 experimental groups.

<table>
<thead>
<tr>
<th>Group</th>
<th>Control</th>
<th>$F$ Treatment</th>
<th>Control</th>
<th>$F$ Treatment</th>
<th>Control</th>
<th>$F$ Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>&lt; 0.000</td>
<td>0.003</td>
<td>0.456</td>
<td>0.263</td>
<td>0.202</td>
<td>0.060</td>
</tr>
<tr>
<td>B</td>
<td>0.003</td>
<td>0.117</td>
<td>0.265</td>
<td>0.178</td>
<td>0.057</td>
<td>0.016</td>
</tr>
<tr>
<td>C</td>
<td>&lt; 0.000</td>
<td>&lt; 0.000</td>
<td>-0.806</td>
<td>-0.591</td>
<td>0.280</td>
<td>0.160</td>
</tr>
<tr>
<td>D</td>
<td>0.001</td>
<td>0.06</td>
<td>-0.409</td>
<td>-0.192</td>
<td>0.092</td>
<td>0.028</td>
</tr>
</tbody>
</table>

$\Delta F$ of all 4 experimental groups detected significant ($P < 0.05$) erosion-interval related change of fluorescence. Both the treatment and control sub-groups of Group A and B demonstrated increase in fluorescence (positive coefficients) with erosion interval while Group C and D demonstrated loss of fluorescence (negative coefficients) with erosion interval.
Table 8.3

Multiple regression analysis of $\Delta D$ with erosion interval for the Treatment and Control sub-groups of the 4 experimental groups.

<table>
<thead>
<tr>
<th>Group</th>
<th>Control</th>
<th>$F$ Treatment</th>
<th>Coefficient</th>
<th>$F$ Treatment</th>
<th>Control</th>
<th>$F$ Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0.064</td>
<td>0.346</td>
<td>0.016</td>
<td>0.011</td>
<td>0.0172</td>
<td>0.007</td>
</tr>
<tr>
<td>B</td>
<td>0.991</td>
<td>0.593</td>
<td>0.001</td>
<td>0.005</td>
<td>0.0000</td>
<td>0.003</td>
</tr>
<tr>
<td>C</td>
<td>0.010</td>
<td>&lt;0.000</td>
<td>-0.017</td>
<td>-0.021</td>
<td>0.0722</td>
<td>0.122</td>
</tr>
<tr>
<td>D</td>
<td>&lt;0.000</td>
<td>&lt;0.000</td>
<td>-0.080</td>
<td>-0.078</td>
<td>0.3010</td>
<td>0.209</td>
</tr>
</tbody>
</table>

$\Delta D$ of only Group C and D detected significant ($P < 0.05$) erosion-interval related decrease in the decay of backscattered intensity; Group C (Treatment sub-group, $P < 0.000$; Control sub-group, $P=0.010$) and Group D (Treatment sub-group, $P < 0.000$; Control sub-group, $P< 0.000$).
Comparison of the regression coefficient between the treatment and control sub-groups of each of the three experimental groups was undertaken.

For $\Delta SMC$ and $\Delta F$, it was found that only the treatment and control sub-groups of Group C demonstrated significant difference ($P=0.036$ and 0.042 respectively) while $\Delta D$ did not detect any difference between treatment and control sub-groups of all 4 experimental groups.
8.10. Figures

Figure 8.1

Figure shows the division of the enamel samples into 4 main groups which were further subdivided into 2 sub-groups. It also shows the flow from Phase I to Phase II of the study.
Figure 8.2

Figure shows the graph of the progression of the mean surface microhardness decrease ($\Delta SM C$) with erosion interval of the Treatment Sub-groups of the 4 experimental groups.

At the end of the study period, the mean cumulative percentage decrease of surface microhardness observed for Group A (exposed to fluoride for 4 weeks prior to acid challenge) was $30.33 \pm 5.11\%$ (mean $\pm$ S.E.), for Group B (exposed to fluoride for 2 weeks prior to acid challenge), $39.78 \pm 3.66\%$, for Group C (natural surface, no prior exposure to fluoride), $27.00 \pm 5.28\%$ and for Group D (polished surface, no prior exposure to fluoride), $76.6 \pm 2.83\%$. 

![Graph showing mean surface microhardness decrease over erosion intervals for different groups.](image-url)
Figure 8.3

Figure shows line graphs comparing the mean $\Delta SMC$ (decrease in surface microhardness) between treatment and control sub-groups of each experimental group.

At the end of the study, Group C demonstrated the greatest difference (between the two sub-groups) in decrease of surface microhardness (26.97%) followed by Group B (10.80%), Group D (4.45%) and Group A demonstrated the least difference (2.37%).
Figure 8.4

Figure shows line graphs comparing the mean $\Delta F$ (loss of fluorescence) between treatment and control sub-groups of each experimental group.

Both the treatment and control sub-groups of Group C and Group D demonstrated a loss of fluorescence. However, Group A and B which were exposed to the test or control product for 30 days and 15 days respectively prior to acid challenge, demonstrated an increase of fluorescence throughout Phase II.
Figure 8.5

Figure shows line graphs comparing the $\Delta D$ (decay in backscattered intensity) between treatment and control sub-groups of each experimental group.

Throughout the study period, only 2 groups (Group C and D) which were not subjected to a pre-treatment demonstrated a decrease in the decay of backscattered intensity while the groups that were subjected to pre-treatment (Group A and B) did not.
CHAPTER 9

SUMMARY
The ultimate goal of the studies undertaken and described in this thesis was to seek a detection tool that could be used in clinical trials to evaluate the efficacy of interventions meant to reduce the rate of dental erosion.

The challenges facing clinical trials that attempt to perform this task (anti-erosion) are very different from clinical trials that set out to evaluate the efficacy of anti-caries products. To date, one of the main differences in the nature of challenges is the illusiveness of the clinical presentation of erosive wear. When a cavitated carious lesion is detected, one is confident that the lesion is in fact caused by caries and not by any other pathological lesion. Appropriate intervention could then be implemented and the status of the lesion monitored over time.

However when one detects a wear lesion clinically, except for lesions occurring in patients suffering from gastroesophageal reflux disease, one could only suspect but could not be completely sure that erosion plays a part in the wear facet. As a result, the efficacy of any intervention aimed at reducing or halting an erosion process would not be found effective if erosion in actual fact did not contribute to the wear lesion. Hence it is extremely challenging if not impossible to identify suitable participants for an in vivo clinical trial that aims to evaluate anti-erosion interventions. It would seem that the most non-disputable choice of participants in terms of the aetiology of wear lesions would be gastroesophageal reflux patients. However the rate of erosion in this group of patients is much higher than the rate of erosion caused by dietary acid [Tantbirojn et al., 2012] and care has to be taken when trying to extend the results unto non-GERD conditions. Until a time when a more objective tool is available to detect erosive wear lesions, it would seem almost futile to endeavour on clinical trials that involve subjects with wear lesions of ambiguous aetiology.

An alternative approach would be to pursue the avenue of inducing dental erosion before or along with concurrent / simultaneous treatment or intervention. This study design eliminates the ambiguity issue and produces more uniform level of erosion
across the samples. However, it is also not without its limitations. It has been adopted in many in situ erosion studies where erosion was induced on enamel or dentin slabs embedded in appliance which was in turn worn by subjects for a fixed period of time. Erosion could either induced intra- or extra- orally [West et al., 2011].

From a ethical viewpoint, it is less controversial when erosion was induced extra-orally. This is because the dentition of the participants is not subjected to the erosive challenge. However, the important interaction between the erosive agent and saliva could not be factored into the study design and hence the study design lacks clinical congruity.

When erosion was induced intra-orally, the dentition of the subjects is also exposed to the acidic challenge and hence was also being eroded. This is not ethically permissible especially if the expected degree of erosion is high. Studies that had adopted this study design either chose to monitor the net bulk loss of the samples and drop participants that demonstrated more than 200µm of surface loss from the study and remineralisation intervention put in place [Hooper et al., 2004] or to use a detection method that is sensitive to the very early stages of erosion when surface loss has not occurred and remineralisation of the eroded teeth was possible [Young et al., 2006].

Erosion in situ studies models that adopted the in vivo acid challenge and intervention avenues are study models that simulate as close as possible the in vivo situation. However there is a need to obtain additional enamel samples which are hard to come by nowadays [Laurance-Young et al., 2011]. The introduction of an appliance into the oral environment might also alter the salivary flow rate which in turn affects the rate of erosion. It also poses as an inconvenience and potential hazard to the participants.

It is obvious then that it would be ideal to be able to conduct in vivo clinical trials that has unequivocal erosion lesion on natural teeth as samples, ethically viable (i.e.
sensitive to early lesions), the erosion process occurring in a natural oral environment and does not involve the use of an appliance.

The first step towards the materialisation of the *in vivo* clinical trial described above was to identify novel assessment tools of demineralisation that has the potential for *in vivo* application. Optical methods such as OCT and QLF had been used in varied *in vivo* situations to detect caries and advanced erosion. Hence it was speculated that they could possibly be used for the purpose of the detecting early enamel lesions too.
9.1 The *In Vitro* studies

*In vitro* studies are valuable in laying down the ground work of identifying the strengths, limitations and detection threshold of a potential detection tool before embarking on more expensive clinical studies. It provides data that can be used to define a scheme or blueprint to follow and refine prior to conducting studies in the clinical environment.

9.1.1 Validation Study (Chapter 5)

9.1.1.1 Methodology

The objectives of the *in vitro* validation study conducted (Chapter 5) were to provide information on whether the two optical methods were able to detect early demineralisation and if so, to determine the detection threshold of the techniques. In order to do so, a suitable and established ‘gold standard’ assessment method had to be identified. As discussed by ten Bosch and Angmar-Mansson, [ten Bosch and Angmar-Mansson, 2000], the choice of the ‘gold standard’ is a matter of reasoning and weighing of arguments and not of following a prescribed procedure. In this study, the choice of ‘gold standard’ which the two optical methods were validated upon was surface microhardness as it is an established method for the quantification of mineral loss. Featherstone et al [Featherstone et al., 1983] had demonstrated a direct relationship between volume percent mineral and the square root of the Knoop Hardness Numbers and concluded that microhardness profiles can be used not only as a comparative measure of hardness changes but as a direct measure of mineral loss as a consequence of demineralisation. Surface microhardness was also chosen due to the possibility of it being used in a longitudinal study design as opposed to transverse microradiography (TMR) which is destructive.

However the surface microhardness technique is primarily designed for assessment of samples with flat and polished samples whilst the samples for this study were natural surfaced ones. The use of surface microhardness as gold standard for this study was made possible under the following strict conditions:
I. Choice of teeth with relatively flat surfaces such as the labial surfaces of the upper and lower incisors.

II. Careful selection of an area that is at right angles to the direction of load. This was done by ensuring the aiming beam of the micro indenter was in focus before the load was applied.

III. Identifying the combination of the minimum load and dwelling time that was able to produce a discernible indent at the highest magnification (in this case was 50 x).

IV. Exclusion of asymmetrical indents or indents with one or more indiscernible sides of the rhomboid.

A commercially available orange juice was chosen as the erosive agent in this study because it was the objective of this study to simulate as closely as possible a clinical situation, one that is transferable to a future in situ or in vivo study. The degree of demineralisation induced in this study was small, only involving surface-softening, with no evidence of surface loss or step change of more than 10 microns as observed in the B-Scans of OCT at the end of the erosion interval (Figure 5.10). This again is because the objective of the study was to explore the capability OCT and QLF to detect a degree of erosion that is ethically viable to reproduce in a clinical trial.

A new algorithm for OCT backscattered intensity was explored and used in this study and it was termed the decay of backscattered intensity, $D$. It is the relationship of the attenuation of backscattered light between two optical depths of an OCT A-Scan, represented by the function below,

\[
D = \frac{I_{\text{plateau}}}{I_{\text{superficial}}}
\]
There are several advantages of using this algorithm and they are as listed below:

I. As the algorithm is a ratio of the backscattered intensity between a superficial and a deeper layer. Hence selective and systematic exclusion of the superficial layers which are affected by specular reflection can be carried out as opposed to the empirical method of excluding signals from superficial layers suggested by Amaechi et al [Amaechi et al., 2003].

II. This new algorithm includes in the calculation, the backscattered intensity from a deeper layer which is not affected by the erosion process. The signal from this layer could potentially serve as a reference in place of a coated reference area. This would be especially advantageous in in vivo and in situ situations where maintaining an area of no change is a big challenge. This possibility was subsequently explored in Chapter 6.

9.1.1.2 Results and Discussion

Measurements from all three instruments (surface microhardness, QLF and OCT) demonstrated significant dose responses with erosive challenge (P < 0.05). All three instruments demonstrated a detection threshold of 10 minutes.

Thereafter, surface microhardness demonstrated significant changes after every 10 minutes of erosive challenge. QLF demonstrated significant difference in fluorescence change only at four other erosive intervals (20, 40, 50 and 60 minutes).

As for OCT, various depth combinations were looked into and it was found that depth combination of 150 µm/30 µm and 150 µm/40 µm seemed to be the optimum depths to be used in the measuring of early demineralisation with OCT. These two depth combinations are the levels nearest to the tooth-air interface that showed significant linear regression with erosion interval but were not affected by specular reflection. Both combinations also demonstrated significant difference in decay at three time points.
9.1.1.3 Hypothesis tested and Conclusions

The hypothesis tested in this study was:

QLF and OCT is not able to detect the progression of early enamel erosion *in vitro*.

The Null Hypothesis was rejected and it was concluded that OCT and QLF were able to detect the progression of early enamel erosion.

It was further concluded that

1. The *in vitro* early enamel demineralisation detection threshold of the QLF set up in this study is 10 minutes of erosive challenge with orange juice.

2. The *in vitro* early enamel demineralisation detection threshold of the OCT set up in this study varies with the different depth combination. For $\Delta D$ (150 µm/30 µm), it is 10 minutes of erosive challenge with orange juice.
9.1.2 Study on Reference Methods (Chapter 6)

Results from the validation study (Chapter 5) had demonstrated that both QLF and OCT could be used to detect and monitor in vitro demineralisation changes albeit with varying sensitivity and detection thresholds.

From the results of Chapter 5 also, it was realised that the new algorithm tested for OCT, the decay of backscattered intensity, $D$, could theoretically eliminate the need for a coated reference area for OCT.

The algorithm for loss of fluorescence, $\Delta F$, on the other hand makes it necessary to have a reference area for the period of the study to account for systematic errors and biological variability. However, it was postulated that perhaps by imbibing the exposed eroded area with a medium that has a similar refractive index or close to that of enamel (1.62), the eroded area itself could be used as its own reference. Water was chosen as the medium to be tested as it has a refractive index closer to enamel (1.33) than air (1.0). It was also chosen as it does not react with the eroded surface and has a molecular size small enough to penetrate into the porosity left behind by the erosion process.

The objective of this in vitro study was therefore to compare the water-moistened eroded surface reference technique with a dentine bonding agent coated reference technique. It was aimed to determine whether $D$, the decay of OCT backscattered intensity could be used to detect and longitudinally monitor demineralisation changes.
9.1.2.1 Methodology

The outcome measure for OCT explored in this study were

$D_E$, the decay of backscattered light of the exposed area as described in Chapter 5.

$\Delta D_1$, the percentage difference of decay between the exposed ($D_E$) and non-exposed
($D_{NE}$) areas;

$$\Delta D_1 = 100 \left[ \frac{D_E(t)}{D_E(t_0)} - \frac{D_{NE}(t)}{D_{NE}(t_0)} \right]$$

and

$\Delta D_2$, the percentage difference of decay between the exposed ($D_E$) and moistened-
exposed ($D_{ME}$) areas, is represented by the function;

$$\Delta D_2 = 100 \left[ \frac{D_E(t)}{D_E(t_0)} - \frac{D_{ME}(t)}{D_{ME}(t_0)} \right]$$

The outcome measures for QLF are as follows:

$\Delta F_1$, the percentage loss of fluorescence of the exposed area, with the non-exposed
area as reference;

$$\Delta F_1(t) = 100 \left[ \frac{F_E(t)}{F_E(t_0)} - \frac{F_{NE}(t)}{F_{NE}(t_0)} \right]$$

$\Delta F_2$, the percentage loss of fluorescence of the exposed area, with the moistened
exposed area as reference;

$$\Delta F_2(t) = 100 \left[ \frac{F_E(t)}{F_E(t_0)} - \frac{F_{ME}(t)}{F_{ME}(t_0)} \right]$$
9.1.2.2 Results and Discussion

For QLF, multiple linear regression of both reference methods showed a significant dose response with erosion interval. The non-exposed surface technique showed significant mean percentage loss of fluorescence (P<0.05) from baseline after 10 minutes of erosive challenge (1.33 ± 0.50 (mean ± SE)), while the moistened exposed surface technique showed a significant loss at 30 minutes, (5.47 ± 2.60 (mean ± SE)). \( \Delta F_2 \) manifested larger standard deviations. These large standard deviations could be due to a difference in the diffusion rate of water into the surface porosity in different sample and 10 seconds of waiting time prior to taking a QLF image may not be sufficient for a uniform permeation of water into the porosity of each sample. However, \( \Delta F_2 \) showed similar trend and order of magnitude of fluorescence loss as \( \Delta F_1 \).

For OCT, multiple linear regressions of both reference methods also showed significant dose response but the non-exposed surface technique showed a much stronger relationship than the moistened-exposed technique. With the depth combination of 150 \( \mu \)m/30 \( \mu \)m, both reference techniques showed significant percentage difference of decay (P<0.05) from baseline after 10 minutes of erosive challenge but the moistened-surface technique did not show any more significant difference in decay thereafter while the difference of decay for the non-exposed surface technique continued to be significant up to end of the study.
9.1.2.3 Hypothesis tested and Conclusions

The hypotheses tested in this study were:

1. A water-moistened eroded surface cannot be used as a reference in the *in vitro* longitudinal monitoring of early enamel erosion with QLF.

2. A water-moistened eroded surface reference technique is not as sensitive as the coated surface reference technique in the monitoring of early enamel erosion with QLF.

3. A water-moistened eroded surface cannot be used as a reference in the *in vitro* longitudinal monitoring of early enamel erosion with OCT.

4. A water-moistened eroded surface reference technique is not as sensitive as the coated surface reference technique in the monitoring of early enamel erosion with OCT.

5. A reference area is needed in the longitudinal monitoring of early enamel erosion with OCT *in vitro*.

Null Hypothesis number 1, 2 and 4 were rejected and it can be concluded that

- A water-moistened eroded surface can be used as a reference in the *in vitro* longitudinal monitoring of 60 minutes erosive challenge using QLF.

- A water-moistened eroded surface reference technique is not as sensitive as the coated surface reference technique in the *in vitro* monitoring of early enamel erosion with QLF.

- A reference area is not needed in the longitudinal monitoring of early enamel erosion with OCT *in vitro*. 


9.2 The In Situ Studies

At this juncture where in vitro results showed that the two optical methods were able to detect early enamel demineralisation caused by orange juice, decisions had to be made for the next course of action; whether to delve straight into an in vivo validation of the two or proceed with an in situ validation. Whilst it is generally accepted that the rate of erosion in vivo is less than that induced in vitro due to the protective effect of the pellicle [Hannig et al., 2004; Meurman and Frank, 1991a], there had not been any previous study that had attempted to derive the in vivo erosion rate from in vitro results. Therefore it was difficult to predict the degree of erosion rate that would be induced in vivo and that could be detected by the OCT and QLF set up. It was felt that since there are still many an unknown clinical condition present, an in situ validation is warranted before finally embarking on using the two optical methods in vivo situations.

9.2.1 Validation study (Chapter 7)

A double-blind (blinded to the research participants and the persons responsible for performing the sample analysis) in situ study was carried out on healthy participants wearing appliance that were embedded with human enamel samples.

The primary objective of the study was to evaluate the sensitivity of OCT and QLF in the detection of early enamel erosion in an in situ setting. However, besides the validation of both optical techniques, two secondary objectives were included in the study. They were to evaluate the difference of response to acid challenge between natural-surface and polished surface enamel and to evaluate whether a pre-exposure period to the oral environment affected the rate of erosion.

9.2.1.1 Methodology

Problems identified in the in vitro study and its results had moulded / shaped the study design of this in situ study. Due to the strict conditions under which the microhardness indents were recorded and the need to identify a sufficiently flat surface, surface microhardness measurement was found to be the time limiting assessment method of
the three used. In an effort to pre-identify a suitable area for indentation and hence expediting the measurement procedure, a non-contact profilometer was used to identify a 2 mm x 1 mm area which is within 300µm of height variation. The identified area was then outlined with three laser indents. This method was found to be effective in expediting the surface microhardness technique. In addition to this, a CCD camera was attached to the micro indenter to image the indents from the *in situ* study so that the measurements of the indent could be carried out on a separate occasion, making it possible to take a higher number of images of indents in a limited time frame.

The bonding agent used in the *in vitro* study was a one-step 5th generation bonding agent where the smear layer was modified but not removed during the process of application. Studies have shown that the bond strength of the newer generations of bonding agent is still inferior to the two steps bonding agent where smear layer is removed [Van Landuyt et.al, 2007]. As the period of the *in situ* study was much longer than the *in vitro* study, it was decided that a 2nd generation 2 step bonding agent be used. However, there are indications that the surface of the bonding agent picked up stain as the study progressed and had affected the stability of the coated reference area for QLF. This was demonstrated by the increase of fluorescence of specimens in Group A and Group B (Figure 7.10) which were pre-exposed to the oral environment before being challenged with acid. Perhaps the use of an index matching medium on the eroded surface would be a good alternative to using a coated reference technique. However, although results in the use of water as the index matching medium described in Chapter 6 had shown it to be able to detect the progression of demineralisation *in vitro*, it is less sensitive than the coated reference technique. Other media that do not interfere with the erosion process should be explored.

Building from the *in vitro* study design where only one B-scan was captured per specimen and hence having re-positioning issues, a series of B-scans were captured for this *in situ* study, resulting in a C-scan of a 3.5 mm x 0.5 mm area. This approach
made en-face view of the eroded and coated area possible. The problem of specular reflection still persisted and it was worse with the polished surface specimens. This problem was partially solved by placing the polished specimens in an angle and also by putting in place a thresholding mechanism in the Matlab program. The outcome measure of $D_E$ was used in this study, as results from the in vitro reference methods study had demonstrated its ability to detect progression of early demineralisation.

The main biological and chemical conditions related to dental erosion were reproduced in this in situ model, with the natural formation of salivary pellicle, and the presence of physiologically secreted saliva during the orange-juice-induced erosive challenge. The choice of a two-hour pellicle was made based on the equilibrium between protein adsorption and de-sorption being reached within 2 hrs [Lendenmann et al., 2000] and a two-hour pellicle had been shown to be acid-resistant to a certain degree [Sonju Clasen et al., 1997]. The lower buccal appliance was the location of choice so as to minimise soft tissue wear from the tongue. A previous study has shown the high wear effect of the tongue on hard tissue samples carried with palatal appliances [Azzopardi et al., 2004]

### 9.2.1.2 Results and Discussion

$\Delta F$, percentage loss of fluorescence, of both the natural and polished surface specimens demonstrated significant net loss of fluorescence as erosion progressed. However, the polished surface samples demonstrated less loss of fluorescence compared to the natural surfaced samples, with a cumulative loss of $1.17 \pm 0.60 \%$ compared to $4.18 \pm 0.90 \%$ of the natural surfaced specimens. This is incongruous with the surface microhardness findings where the natural surface specimens demonstrated less decrease in surface microhardness (-46.03 ± 5.05%) than the polished surface specimen (-81.05 ± 2.66 %). This might be because the enamel layer of the polished samples were thinner than the natural surfaced enamel and hence is closer to the amelodentinal junction (ADJ) which is believed to be the main source of fluorescence.
The percentage change of decay in backscattered intensity, $\Delta D$, of both natural and polished specimens showed significant decrease as erosion progressed. The relative degree of changes between the two groups of specimens were in agreement with those of surface microhardness, with the natural surface specimen demonstrating less change ($11.87 \pm 3.51\%$) than the polished surface specimen ($40.57 \pm 5.58\%$).

When comparing OCT and QLF, Pair t test results indicate that, similar to the results of the in vitro study, and under this in situ setting, QLF was more sensitive than OCT in the detection of demineralisation changes on natural surfaced enamel. However, the opposite is true for polished-surface enamel.

The regression coefficients of percentage of surface microhardness change ($\Delta SM C$), of the three natural surface groups were compared to assess whether their decreases were significantly different. Although the group that was exposed to the oral environment for 30 days showed less decrease of surface microhardness compared to the other two groups of natural surface specimens, it was found that $\Delta SM C$ of these three groups were not significantly different ($P > 0.05$) from each other.

### 9.2.1.3 Hypothesis tested and Conclusions

The Null Hypotheses tested in this study were:

1. QLF is not able to detect and longitudinally measure early enamel erosion on natural- surface enamel in situ.
2. OCT is not able to detect and longitudinally measure early enamel erosion on natural- surface enamel in situ.
3. QLF is not able to detect and longitudinally measure early enamel erosion on polished- surface enamel in situ.
4. OCT is not able to detect and longitudinally measure early enamel erosion on polished- surface enamel in situ.
5. Polished-surface enamel and natural surface enamel does not erode in the same rate.

6. Pre-treatment of samples in the oral environment prior to erosion does not affect the rate of demineralisation.

All null hypothesis were rejected and it can be concluded that

- OCT and QLF were able to detect and longitudinally measure in situ demineralisation in natural samples subjected to 150 minutes of in vivo acid challenge with orange juice.
- OCT and QLF were able to detect and longitudinally measure in situ demineralisation in polished samples subjected to 150 minutes of in vivo acid challenge with orange juice.
- Polished surfaces eroded faster than natural surfaces in the conditions of the study.
- Pre-treatment of samples in the oral environment prior to erosion does not affect the rate of demineralisation.
9.2.2 Effect of a High Fluoride Dentifrice on Early Enamel Erosion

(Chapter 8)

This study was part of the study described in Chapter 7. It was a single centre, double blind (blinded to the research participants and the persons responsible for performing the sample analysis), randomised, 2 product (1 test and 1 control), two treatment phase, split mouth, in situ study. The test product used was Duraphat 5000ppm (Colgate-Palmolive, Guildford, United Kingdom) and the control used was deionised water.

The primary objective of the study was to evaluate whether there was a protective effect of treating human enamel with Duraphat 5000ppm during an active erosion phase. However besides evaluating the efficacy of the dentifrice, there were two secondary objectives and they were to evaluate whether there was a protective effect of pre-treating human enamel with a Duraphat 5000ppm prior to the onset of acid challenge and to evaluate whether there is a difference in the efficacy of Duraphat 5000ppm in reducing the rate of early erosion between polished and natural surface human enamel.

Oral health products had traditionally been used as a secondary preventive agent against erosion. They were hardly tested as a primary preventive agent. Primary prevention is directed towards preventing the initial occurrence of a disorder [WHO, 1998]. The rationale of introducing a pre-erosion treatment phase was to evaluate whether Duraphat 5000ppm could be used as a primary prevention agent against erosion. There was also equally scarce research conducted to discern the relative susceptibility of natural and polished surfaces to erosive challenge and responses to preventive therapies. Most conclusions of reported studies on the efficacy of preventive products against erosion had derived conclusions from using polished enamel as a substrate.
9.2.2.1 Methodology

There are significant variation in terms of intra- and inter- teeth surface hardness, hence attempts have to be made using the same tooth for both treatment and control sub-groups to minimize biological variations. Meurman and Frank [Meurman and Frank, 1991b] observed great morphological variations in erosive lesions in specimens prepared from the same human tooth. Effects due to sample preparation and variations between different teeth have to be taken into account in future studies. If natural enamel surfaces are used, significant variations between samples prepared from the same tooth, as well as from different teeth can be expected [Ganss et al., 2000]. The removal of the surface layer provides samples of more consistent composition when prepared from the same tooth, but significant variations between different teeth must be taken into account if specimens are divided into groups. The sample size calculations were carried out based on Hooper et al's study [Hooper et al., 2004] where they had used polished samples. Judging from the variation observed in these in situ studies, a larger sample size might have been necessary to detect significant differences in results.

9.2.2.2 Results and Discussion

Multiple regression analysis of $\Delta SM_C$ with erosion interval of both Treatment and Control sub-groups for all 4 groups showed that surface microhardness detected significant ($P < 0.05$) erosion-interval related decreases. Only the treatment and control subgroup of the natural surface specimens (Group C) demonstrated significant differences ($P=0.036$) while those of pre-treated natural surface groups (Group A and B) as well as those of polished-surface group did not ($P=0.248$). Pre-treating specimens with the high fluoride dentifrice did not seem to provide any anti-erosion effect. The results of Group D (polished surface) obtained in this study concurs with other previous studies that evaluated the anti-erosion effects of sodium fluoride dentifrice at neutral pH [Magalhaes et al, 2007; Rios et al, 2008].
Multiple regression analysis of $\Delta F$ with erosion interval for all 4 groups showed significant ($P < 0.05$) erosion-interval related changes of fluorescence. Group C was the only group that showed significantly different regression coefficients between the Treatment and Control sub-groups. This result concurs with those of surface microhardness.

$\Delta D$ however did not detect any difference between treatment and control sub-groups in all 4 experimental groups. It is postulated that the presence of oral biofilm confounds the OCT backscattered intensity as some recent studies had reported using OCT to image ex vivo biofilms on dental composites and on orthodontic brackets and assess their thickness [Garcez et al, 2011; Lenton et al, 2012].

9.2.2.3 Hypothesis tested and Conclusions

The Null Hypotheses tested were:

1. Pre-treating natural surface enamel with a high fluoride dentifrice regime does not increase the resistance of enamel against $in vivo$ erosive challenge regime with an orange juice.

2. Treating natural surface enamel with a high fluoride dentifrice does not increase its resistance against concurrent early $in vivo$ erosion with an orange juice challenge regime.

3. Treating polished-surface enamel with a high fluoride dentifrice does not increase its resistance against early $in vivo$ erosion with an orange juice challenge regime.

4. There is no difference in the effect of treating natural surface or polished surface enamel with a high fluoride dentifrice.

5. QLF is not able to detect the protective effect of a high fluoride dentifrice on natural or polished surface enamel against early $in vivo$ erosion with an orange juice challenge regime.

Null Hypothesis numbers 2 and 4 were rejected and hence it can be concluded that
1. Treating natural surface enamel with a 5000ppm NaF dentifrice increased its resistance against concurrent early *in vivo* erosive challenge with an orange juice challenge regime.

2. QLF was able to detect the protective effect of a 5000ppm NaF dentifrice on natural surface enamel against early *in vivo* erosion with an orange juice challenge regime.


9.3 Future Work

Although both OCT and QLF are optical methods that have been employed in numerous dental caries and erosion studies, to date they have not been used to assess early enamel erosion.

Not only have the use of OCT and QLF been explored in assessing early enamel erosion, there are also other novel ideas and methods explored within the *in vitro* and *in situ* studies described in this thesis and they are as listed below:

1. The proposal of a new algorithm to analyse the OCT backscattered intensity, which is $D$, the relationship of the attenuation of backscattered light between two optical depths (Section 5.4.4)

2. The technique of using surface microhardness on natural surface enamel (Chapter 4).

3. The proposal of using the eroded surface itself, imbibed with an index-matched medium as a reference method (Chapter 6).

4. Parallel comparison of the erosion rate of polished and natural surface enamel and the different effect a high fluoride dentifrice has on these two substrates.

5. Exploration of the potential of a high fluoride dentifrice as a primary preventive agent.

6. A bespoke QLF set up was used.

As the above listed ideas and methods are new, more work is needed to explore all the above listed in greater depth.

9.3.1 Natural vs polished surface specimen

The results of the *in situ* studies described in this thesis (Chapter 8) suggest that further research on natural, unpolished enamel samples is necessary to help elucidate in more clarity the effects of polishing on demineralisation under erosive conditions and
re-mineralisation effects of preventive agents. This echoes Lippert et al’s recommendation [Lippert et al., 2004].

9.3.2 OCT

9.3.2.1 Assessment of Early Erosion with OCT

With specular reflection confounding signals from the enamel-air interface to about 50 μm physical depth below (30 μm optical depth), important information of the degree of demineralisation within those layers with erosion time is lost. Similar work should also be carried out with Polarised-Sensitive Optical Coherence Tomography, as it has been reported that this type of OCT is designed to be able to detect only orthogonally polarised light and hence is not compounded by specular reflection [Jones and Fried, 2006].

9.3.2.2 Comparison of the algorithm of OCT outcome measure

Further work is needed to compare the algorithms explored in the in vitro study (Section 6.5.2) with those which were used by Jones and Fried [Jones and Fried, 2006] for the quantification of artificial smooth surface caries and Amaechi et al [Amaechi et al., 2001] and Popescu et al [Popescu et al., 2008] for early erosion.

9.3.2.3 Assessment of Erosive Wear with OCT

Exploration into the sensitivity of using OCT in the assessment of erosive tooth loss is also needed, although Wilder-Smith et al [Wilder-Smith et al., 2009] have used this capability of OCT in a 3-weeks clinical trial to evaluate the efficacy of an acid-suppressive treatment.

Like the contradictory results of the efficacy of anti-erosion oral health products, many epidemiological studies had failed to demonstrate any strong correlations between the prevalence of erosion and potential risk factors such as acidic beverages or diet or medication. These results could again be due to the lack of a robustly validated clinical
index for dental erosion and to the absence of an objective clinical detection tool that could be used to validate these indices.

9.3.2.4  Assessment of Dentine erosion with OCT

These *in vitro* studies should also be extended to evaluate the capability of OCT in the detection of erosion in dentine.
9.4 References


APPENDIX 1 - Patient Information Sheet for the *In situ* Study

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RESEARCH PARTICIPANT INFORMATION SHEET  
A Study Investigating the Detection of Dental Erosion Using 3 Optical Methods  
Protocol Number – NW-COL-01

You are being invited to take part in this research study. Before you decide whether or not to take part, 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. Part 1 tells you the purpose of the study and what will happen to you if you take part. Part 2 gives more detailed information about the conduct of the study. Ask us if there is anything that is not clear or if you would like further information. Please take time to decide whether or not you wish to take part. Thank you for your interest in this research study.

**PART 1**

**What is the purpose of this study? Why is this study being carried out?**

Colgate-Palmolive (UK) Ltd. is the sponsor and funder for this study. This means that they are responsible for design, management and financing of this study.

Dental erosion is defined as a chemical process that involves the dissolution of enamel and dentine by acid which is not derived from bacteria.

The aim of this study is to investigate the sensitivity of 3 optical measuring techniques, Optical Coherence Tomography (OCT), Quantitative Laser Fluorescence (QLF) and non-contact profilometry, in detecting early dental erosion. The sensitivity of the OCT and QLF optical methods will be compared to the more established technique of measuring erosion using non-contact profilometry.

The enamel samples to be used in the study are made from healthy human incisor teeth that are donated by healthy patients aged over 18 years. The teeth are thoroughly sterilised prior to use. Teeth are held in the ethically approved Bristol Dental School and Hospital Tooth Tissue Bank until requested for use in a study.

**Why have I been chosen?**

You have been chosen because you meet the study entry criteria of being at least 18 years of age. Altogether, a maximum of 20 healthy subjects will take part in this study, which will last
approximately 10 weeks. The study has been approved by an independent ethics committee (Devon and Torbay Research Ethics Committee – REC Ref: 09/H0202/60) and is being funded by Colgate-Palmolive (UK) Ltd. All potential participants will work or study within Bristol Dental School and Hospital.

Do I have to take part in this study?

NO. You are entirely free to choose and you may stop taking part in the study at any time without giving a reason. If you do decide to take part, you will be asked to sign a consent form, of which you will be given a copy along with this information sheet to keep.

Although unusual, the dentist also has the right to stop your participation in this study in the event of you experiencing illness, or for administrative or other reasons.

We will also keep you informed of any new information that may become available during the trial that may affect your willingness to continue participation in the trial.

What will happen to me if I take part in this study / what will I have to do?

If you agree to participate in this study, you must read and sign this Research Participant Information Sheet and Consent Form before any study procedures begin. You will be given a copy of this Research Participant Information Sheet and Consent Form to keep.

There will be a screening visit and two treatment phases; the first being 6 weeks in duration (weekdays only), the second will be 2 weeks (5 treatment days on alternate days, weekdays only). There will be a follow up visit within 2 weeks of the end of treatment phase 2.

To complete this study you will need to attend the study site on 112 occasions over approximately 10 weeks. The time commitment to the study will be as follows:

- Screening visit: Approximately 15 minutes at the study site, this will include taking an impression of your mouth so that we can make two lower mouth appliances for you (if required).

- Two treatment phases. Treatment phase 1 will be 30 days in duration; treatment phase 2 will be 5 days in duration (alternate days over a two week period).

- **TREATMENT PHASE 1**: Two lower mouth appliances (each containing up to 4 enamel samples) will be worn from 09:00 ± 30 minutes to 16:30 ± 30 minutes on each treatment day. The appliances will be worn for a minimum of 5 hours per day. Removal of the appliances will be a minimum of 60 minutes after the final treatment of the day.

  There are 2 treatments per day at 09:00 ± 30 minutes and 15:30 +/- 30 minutes. You will be required to attend the study site at these times for approximately 5 minutes on each occasion. The appliances will be removed from your mouth and treated by soaking them in a slurry of the allocated product for 2 minutes. The appliances will then be returned to your mouth.

  Your appliances must be returned to the study site at least 1 hour after the last treatment of the day (16:30 +/- 30 minutes).

  The appliances should be worn for at least 6 hours per day.

- **TREATMENT PHASE 2**: As in treatment phase 1, you must attend the site twice per day for treatment application (same process as in Treatment phase 1) at 09:00 +/- 30 minutes and 15:30 +/- 30 minutes.

  Immediately after the first treatment application, the appliances will be placed into your mouth and you will be required to swish 25ml of orange juice in your mouth for one minute, SPIT OUT THE ORANGE JUICE, then immediately begin swishing with 25ml of orange juice for another minute. You will repeat this until you have swished for a total
of 10 minutes, thereby exposing the enamel samples to 250 ml of the orange juice over a 10 minute period. A second orange juice challenge will occur at 13:00 +/- 30 minutes. A third orange juice challenge will occur at 15:30 +/- 30 minutes, immediately before the second treatment application, totalling 2 treatment applications and 3 acid challenges per day.

Your appliances must be returned to the study site at least 1 hour after the last acid challenge of the day (16:30 +/- 30 minutes).

- Follow up visit: 10 minutes at study site.

Screening visit

This may take place up to 1 month before your first treatment day. At screening your gender, date of birth and ethnicity will be recorded and a suitably qualified member of staff will take a medical history from you. The dentist will examine your mouth. Assuming you meet the study eligibility criteria, if a current study model of your lower teeth is not available, we will take a mould of your lower teeth once we have established your eligibility to be included in the study. You will be informed of the start date for the trial and given a standard, commercially available toothbrush and dentifrice which you must use at home for tooth cleaning, rather than using your normal oral hygiene / tooth cleaning practices, twice daily throughout the study until your follow up visit. If you already have existing appliances available, they may be tried for fit during this visit.

**Example of a lower left mouth appliance**

**Example of lower left and right mouth appliances**

**Study treatment days**

Your existing or new appliances will be tried for fit if applicable, and assuming you are eligible to continue, you will be entered onto the study.

This study will consist of 2 treatment phases. At the start of treatment phase 1, each appliance will be fitted with 1 enamel sample. After 10 15 days of treatment, another enamel sample will be added. After a further 10 days of treatment another enamel sample will be fitted into each appliance. At the start of treatment phase 2 an additional of 2 enamel sample will be added so that there are 4 samples in each appliance for the commencement of treatment phase 2.

During each treatment period, different study products will be applied to the samples in the lower left and right mouth appliances. Over the course of the two treatment phases your enamel samples will be treated to both of the study products, one product on each appliance (left or right side of your mouth) throughout the study. This will be decided randomly using a randomisation schedule provided by Colgate-Palmolive (UK) Ltd.

**TREATMENT PHASE 1** (30 days, excluding weekends):

At the start of each study day, prior to the insertion of the appliances into your mouth, both appliances will be disinfected. You will then be asked to place them in your mouth. The
appliances will be worn from 09:00 ± 30 minutes to 16:30 ± 30 minutes. The appliances will need to be removed for up to a 1 hour period over lunch and may also be temporarily removed, by yourself, prior to eating or drinking anything at other points during the study day. We will provide you with a pot to keep the appliances in when they are out of your mouth. During each study day you will be required to attend the study site on two occasions so that applications of the study treatments can be made by one of the clinical trials team. You will be required to return the appliance at the end of the study day.

The times for attending the study site are:

<table>
<thead>
<tr>
<th>Procedure/ Time clock</th>
<th>Before 07:30</th>
<th>Guide time of 08:00 - 08:30</th>
<th>09:00 (± 30 min)</th>
<th>13:00 (± 30 min)</th>
<th>15:30 (± 30 min)</th>
<th>16:30 (± 30 min)</th>
<th>After 21:00</th>
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<tbody>
<tr>
<td>Brush teeth at home</td>
<td>X</td>
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<tr>
<td>Disinfect appliances and/or enamel samples</td>
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<td>Appliances inserted into mouth</td>
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<td>Treatment Application</td>
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<tr>
<td>Removal of appliance / Food Intake</td>
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<td>Appliances removed from mouth</td>
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**TREATMENT PHASE 2 (5 days):**

Due to the time required to perform the measurements on the enamel samples, for this treatment phase you will receive treatment on alternate days over a 2 week period (Weekdays only).

As in Treatment Phase 1, you will be required to wear the intra-oral appliances from 9:00 +/- 30 minutes until 16:30 +/- 30 minutes and must attend the study site twice per day for treatment application at 09:00 +/- 30 minutes and 15:30 +/- 30 minutes.

Immediately after the first treatment application, the appliances will be placed into your mouth and you will be required to swish 25ml of orange juice in your mouth for one minute, SPIT OUT THE ORANGE JUICE, then immediately begin swishing with 25ml of orange juice for another minute. You will repeat this until you have swished for a total of 10 minutes, thereby exposing the enamel samples to 250 ml of the orange juice over a 10 minute period. A second orange juice challenge will occur at 13:00 +/- 30 minutes. A third orange juice challenge will occur at 15:30 +/- 30 minutes, immediately before the second treatment application, totalling 2 treatment applications and 3 acid challenges per day.

The appliances will be returned to the study site at least 1 hour after the last acid challenge of the day. Prior to performing any measurements on the enamel samples, the appliance with the enamel samples in will be disinfected with a 70% ethanol solution for at least 2 minutes. Samples will then be removed from the appliances and wiped with Chlorhexidine Gluconate to remove any plaque that may have formed.

<table>
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<tr>
<th>Procedure/ Time clock</th>
<th>Before 07:30</th>
<th>Guide time of 08:00 - 08:30</th>
<th>09:00 (± 30 min)</th>
<th>13:00 (± 30 min)</th>
<th>15:30 (± 30 min)</th>
<th>16:30 (± 30 min)</th>
<th>After 21:00</th>
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<tbody>
<tr>
<td>Brush teeth at home</td>
<td>X</td>
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<td>X</td>
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<td>Treatment Application</td>
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<td>Appliances inserted into mouth</td>
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</table>
Acid Challenge

| Appliances removed from mouth | X | X | X | X |

Please ensure that the appliances are worn in your mouth for at least 6 hours. Please also take into account the time they are out of your mouth for lunch etc.

For application of the study treatment to occur, you will be asked to remove the appliances from your mouth so that the whole appliance, including the enamel samples, can be placed into a glass beaker containing the allocated dentifrice slurry. At the end of each study day, the appliances will be removed from your mouth and again disinfected, then stored appropriately overnight in a numbered, moist pot.

Each day you will be asked about any medications you may have taken or if you have experienced any adverse events since your last visit.

At the end of each study day during treatment phase 2, the enamel samples will be removed from each appliance for analysis by 3 optical measuring techniques.

Follow up visit

This will take place within 2 weeks of your last treatment day. During this visit the dentist will examine your mouth to ensure that you have not suffered any side effects from participating in the study. You will be asked about any medications you may have taken or if you have experienced any adverse events since your last visit.

Expenses and Payment

After the follow-up examination, at the end of this study you will receive £600 for your participation and to cover any out-of-pocket expenses. Depending on your status, Bristol University may deduct tax from this amount, at the basic rate, prior to you receiving payment. If this is not the case, the money obtained from this study is subject to tax and participants must declare it as such. If for any reason you do not complete the study, the sum you receive will be pro rata.

Is there anything I should or should not do?

Once you have agreed to participate in this study, you will be asked to use a standard toothbrush and dentifrice to brush your teeth twice a day (before 8:00am in the morning and after 9:00pm in the evening) throughout the study until you return for the follow up visit. The standard toothbrush and dentifrice will be given to you at the screening visit. You will need to keep any empty tubes and return them at the end of the study. You will be asked not to use any other oral health products such as mouth rinses, dentifrices or toothbrushes throughout the study. You are permitted to use dental floss if it is part of your normal routine.

During treatment days, you are allowed to drink tea, coffee or water but the appliances must be removed beforehand (only water can be consumed whilst the appliances are in place) and placed in the moist pot we will provide. You must abstain from eating, smoking and chewing gum while wearing the appliances. Although there is no restriction on the types of food that can be consumed when you are not wearing the appliances, no other drinks, including acidic beverages such as lemon or herbal teas and fruit flavoured spring water and carbonated beverages are permitted on study days.

During study days, you will be asked not to take any acidic medication (pH<5.3), antacids or vitamin C preparations as they could potentially promote enamel wear.

**IF YOU TAKE ANY MEDICATION IN ADDITION TO THOSE RECORDED AT YOUR SCREENING VISIT, INCLUDING AT THE WEEKEND YOU MUST INFORM US.**

This will be recorded in your case report form.

If you subscribe to a private dental healthcare plan, then you are advised to inform the providers of the plan about your participation in the study incise it voids your cover.
What are the products being tested?

There are 2 treatment possibilities. You will receive both treatments at random (by chance, like flipping a coin) during the study. The randomisation process will determine whether it will be applied to the left or right appliance. The treatments are:

1. 3g of Colgate Duraphat5000 dentifrice mixed with 30ml of deionised water to make a slurry
2. 30ml of deionised water

What are the possible risks of taking part?

There may be a small degree of discomfort when you first start wearing the oral appliances. Should this be the case, please notify the study staff who will adjust the appliance accordingly. All human enamel tooth specimens placed in the mouth have been sterilised appropriately. There is an extremely remote risk of transmission of prions (microscopic protein particles) to you as a result of taking part in the study, but this is no more than the risk when you have routine dental treatment.

It is a risk that some erosion of your natural teeth will occur with the orange juice drink; however, this risk will be extremely small. Nevertheless, for your safety, a maximum level of erosion of 20 microns (0.02mm) has been set with regards to the enamel samples held within the palatal appliance. If upon measurement readings of the surface profile of your enamel samples reach this level during the study, you will be removed from the treatment period. A 20 micron loss of tooth structure is so small it cannot be detected by clinical observation.

Are there any side effects?

You are not expected to experience any side effects from the dentifrice formulations being assessed in this trial or from the standard dentifrice provided. Since there is always the possibility that a rare or previously unknown side effect may occur in somebody using a dentifrice that they may not have not used before, trained dental staff are available in the Clinical Trials Unit.

Are there any benefits in taking part?

There is no direct, immediate benefit to you from taking part in this research study. However, you may have helped us to develop new measuring techniques to detect early dental erosion.

Are there any reasons why my participation in this study could be ended?

The following are reasons why you may be asked to withdraw from the study:

Your safety (such as an adverse reaction)
At your request
If you don’t comply with the study procedures
The Investigator’s request

What happens when the research study ends?
When the study has finished, you must return the used standard toothbrush and dentifrice to the study site and you can then resume your normal oral hygiene routine.

Approximately 3 months after the study has finished, a summary of the results will be available for you to collect from the Clinical Trials Office if you so wish.

**What if there is a problem?**

Any complaint about the way you have been dealt with during the study or any possible harm you might suffer will be addressed. The detailed information on this is given in Part 2.

**Will my taking part in the study be kept confidential?**

YES. All the information about your participation in this study will be kept confidential. These details are included in Part 2.

**Contact details**

If you have any further questions concerning the study, or in case of any difficulty during the study please contact:

**Primary Contact:**
Dr. Nicola West  
Department of Oral & Dental Science  
Bristol Dental Hospital & School  
Lower Maudlin Street  
Bristol BS1 2LY  
Tel: 0117 342 4505

**Secondary Contact:**
Amy North (Study Coordinator)  
Clinical Trials Department  
Bristol Dental Hospital & School  
Lower Maudlin Street  
Bristol BS1 2LY  
Tel: 0117 3423561/4333

This completes Part 1 of the Information Sheet.

If the information in Part 1 has interested you and you are considering participation, please continue to read the additional information in Part 2 before making a decision.

**PART 2**

**What if relevant new information becomes available?**

Sometimes during the course of a research project, new information becomes available about the treatment that is being studied. If this happens, your research dentist will tell you about it and discuss whether you want to or should continue the study. If you decide to continue in the study you will be asked to sign an updated consent form. Also, on receiving new information your research dentist might consider it to be in your best interests to withdraw you from the study and he/she will explain the reasons why. If the study is stopped for any other reason, you will be informed why.

**What happens if something goes wrong?**

If you are harmed due to someone’s negligence, then you may have grounds for a legal action but you may have to pay for it. Regardless of this, if you wish to complain, or have any concerns about any aspect of the way you have been approached or treated during the course of this study, please contact Dr Nicola West, the Principal Investigator of this study.

If you experience any side effects that are study related, which is unlikely, compensation for any injury caused will be in accordance with the guidelines of the Association of the British Pharmaceutical Industry (ABPI). Broadly speaking the ABPI guidelines recommend that ‘the sponsor’ (Colgate-Palmolive (UK) Ltd.), without legal commitment, should compensate you without you having to prove that it is at fault. This applies in cases where it is likely that such injury results from giving any new drug or any procedure carried out in accordance with the...
protocol for the study. ‘The sponsor’ will not compensate you where such injury results from any procedure carried out which is not in accordance with the protocol for the study. Your right at law to claim compensation for injury where you can prove negligence is not affected. Copies of the ABPI guidelines are available on request.

**Will my taking part in this study be kept confidential?**

The results will be analysed by Colgate-Palmolive (UK) Ltd. the company conducting the trial, and in due course may be reviewed by the regulatory and claims approval authorities. This may involve inspection of all study-related information but your identity will always remain strictly confidential. Your name or initials will not appear on any information collected.

A representative of Colgate-Palmolive (UK) Ltd. may observe the study procedure at 1 or more of your study visits.

For the purposes of the Data Protection Act the investigator fulfils the prescribed role of data controller. Your data will be made available to the sponsor’s employees, investigators, members of the ethics committee and/or employees of the competent supervisory authorities exclusively in anonymised form for the purposes of examining data. Your participation in the study will be treated as confidential, that is, any personally identifiable information will be held and processed under secure conditions at Colgate (or an agent of Colgate-Palmolive (UK) Ltd.) with access limited to appropriate Colgate-Palmolive (UK) Ltd. staff or other authorised agents having a requirement to maintain the confidentiality of the information. Data will be kept on file for 15 years. You will not be referred to by name in any report or publication (in a scientific journal) of the study. Your identity will not be disclosed to any person, except in the event of a medical emergency or if required by law. You may be entitled under law to access your personal data and to have any justifiable corrections made. If you wish to do so, you should request this from the investigator conducting the study

Your anonymised data will be processed electronically to determine the outcome of this study, and to provide it to health authorities/drug regulatory agencies. Your data, which is anonymised, will not be used for any other research. No request will be made to look at your dental and medical records. A representative of Colgate-Palmolive (UK) Ltd. may observe the study procedures at one or more study visits.

If you consent to take part in this study we will notify your dentist (‘general dental practitioner’) of your participation.

**What will happen to the results of the research study?**

It is possible that the results of the study will be published in an internationally refereed scientific journal. Should this be the case any information about you will be anonymised as detailed in ‘Confidentiality’ above.

**Who has reviewed the study?**

This study has been reviewed and given favourable ethical approval by Devon and Torbay Research Ethics Committee.

**Intellectual property statement:**

The information and any materials or items that you are given about or during the study (such as information regarding the study drug(s) or the type of study being performed) should be considered the confidential business information of the study sponsor. You are, of course, free to discuss with your friends and family while considering whether to participate in this study or at any time when discussing your present or future healthcare.

Thank you for your help, if you have any further questions, please do not hesitate to ask.
APPENDIX II - CONSENT FORM

University of
BRISTOL
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Lower Maudlin Street, BRISTOL BS1 2LY
Tel: +44 117 3424505
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Dr N West BDS FDS RCS PhD FDS (Rest Dent)
Senior Lecturer/ Honorary Consultant in Restorative Dentistry
E-mail: N.X.West@bristol.ac.uk

RESEARCH PARTICIPANT CONSENT FORM

A Study Investigating the Detection of Early Dental Erosion Using 3 Optical Methods
Protocol Number – NW-COL-01

<table>
<thead>
<tr>
<th>Please initial boxes</th>
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<tbody>
<tr>
<td>I confirm that I have read and understood the information sheet dated 9th October 2009, Version 3.0 for the above study and have had the opportunity to ask questions</td>
</tr>
<tr>
<td>I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical/dental care or legal rights being affected.</td>
</tr>
<tr>
<td>I give permission for my General Dental Practitioner ‘Dentist’ to be notified of my participation in the study.</td>
</tr>
<tr>
<td>I give permission for my General Medical Practitioner ‘Doctor’ to be notified of my participation in the study if necessary.</td>
</tr>
<tr>
<td>I agree to take part in the above study.</td>
</tr>
</tbody>
</table>

Participant Screening Number

____________________________  ________________  ______________
Signature of Participant     Full name of Participant (print)     Date

____________________________  ________________  ______________
Signature of Person Taking Consent  Name of Person Taking Consent (print)  Date