THE EFFECT OF FLUORIDE GLASS SLOW-RELEASE DEVICES ON THE PROTECTION OF PRIMARY AND PERMANENT DENTAL ENAMEL TO EROSIVE CHALLENGE

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

Dedicated to my Family

(MY PARENTS AND MY SIBLINGS)

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Abstract

Aim: To investigate the use of fluoride glass slow-release devices (FGSRD) for the prevention of dental erosion of human dental enamel in vitro.

Methods: Human teeth (permanent and primary) were used for this study. Enamel slabs were randomly allocated to four study groups: Group 1: 24 permanent enamel slabs with FGSRD, Group 2: 24 permanent enamel slabs with placebo non-FGSRD, Group 3: 20 primary enamel slabs with FGSRD, Group 4: 20 primary enamel slabs with placebo non-FGSRD. The glass slow-release devices were randomised into two groups. Test and placebo groups were coded until the end of the study. The enamel slabs were dipped in a citric acid solution for two minutes five times daily for 28 days and brushed twice a day. This was to create the erosive environment for this in vitro study. The slabs were kept in artificial saliva and stored in an incubator at 37^{o}C. The glass slow-release devices (fluoride and non-fluoride) were present in all containers. The surface profile was measured at baseline using surface profilometry and after 14 and 28 days of the cycling regime.

Analysis: Simple t-tests were used to compare the permanent and primary teeth groups with 0.05 as the significance level and an ANOVA t-test with a Bonferroni correction to compare: primary and permanent teeth. Daily fluoride release of the FGSRD's was measured.

Results: For enamel of primary teeth, after 14 days 40% less erosion was observed in the F group which decreased to 31% at the end of the study period, i.e., 28 days. This was highly statistically significant ($p<0.001$) at both time points. For permanent enamel, no significant differences were observed ($p=0.091$).

Conclusion: FGSRD's have great potential for protection of primary human enamel against erosive challenge in addition to a number of other uses.

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List of Abbreviations

F	Fluoride
FGSRD	Fluoride glass slow release devices
g	Grams
h	Hour
µm	Micrometres
mm	Millimetres
min	Minutes
Non-FGSRD	Non-Fluoride glass slow release devices
ppm	Parts per million
sec	Seconds
SE	Standard error
SL	Surface loss
TISAB	Total Ionic Strength Adjustment Buffer

1 Introduction

Erosion is the irreversible tooth tissue loss due to chemicals and not due to bacteria as happens in dental caries. Erosion has been one of the common reasons for dental tooth structure damage in developing countries. The effects and causes of erosion have been thoroughly investigated. There is an abundance of products such as soda drinks and juices that can lead to tooth surface loss in permanent and primary dentitions.

Children and adults are diagnosed with erosion but not until it has progressed and tooth surface loss is clearly visible. It can cause problems such as fractures of teeth or even sensitivity.

Developing in vitro models simulating physiological conditions is an essential part of dental research. Researching the effects of the materials in the controlled environment of a laboratory is the first step to test a material before it can be used in dental practice.

Developing and using an in vitro erosive challenge can replicate the conditions that can lead to erosion in human teeth without endangering anyone.

Fluoride glass slow-release devices (FGSRD) have been tested and proven beneficial against caries and hypersensitivity. The majority of these studies were mainly against caries and reducing hypersensitivity and some investigating the prevention of white spot lesions during orthodontic treatment.

However, to date, there has not been any study using the FGSRDs to investigate their effect on dental erosion.

In this research project, the effect of FGSRD has been investigated in an erosive trial in vitro.

2 Literature Review

2.1 Dental Surface Loss

2.1.1 Mechanism of dental erosion

Enamel is the hard, protective coating of the tooth, which protects the sensitive dentine underneath. When the enamel is worn away, the dentine underneath is exposed, which may lead to pain and sensitivity.

When the surface is worn due to chemical action and not bacterial action this is known as dental erosion. Attrition may be defined as direct tooth-to-tooth contact wear, whilst particles moving across and contacting the tooth surface results in abrasion. Erosion usually co-exists with attrition and/or abrasion, but one of these factors may be more significant than the others making the differential diagnosis difficult (Imfeld, 1996).

The dental enamel in the oral environment is covered by pellicle, a layer of organic material composed of salivary proteins and glycoproteins (Hannig et al., 2005). Erosive solutions destroy first the pellicle and then they interact with the tooth surface, where the enamel crystals are dissolved by the hydrogen ions and give the tooth a honeycomb appearance (Meurman and Frank, 1991). The un-ionised particles of acid then defuse the minerals of inter-prismatic areas (Featherstone and Rodgers, 1981). This leads to a release of calcium and phosphate ions that causes the pH to rise in the subsurface region (Lussi and Hellwig, 2001).

Hydrogen ions of acids bind the calcium in enamel that leads to erosion, it binds with either carbonate ions or the phosphate ions and dissolves them (Featherstone, 2000).

Citric acid and similar acids follow two different chemical pathways in dental erosion. When they are prepared in water they release hydrogen ions, citrate anions, other anions and un-dissociated acid molecules. The amount of each ion is determined by the acids pH and their equilibrium constant. The second chemical pathway is that of citrate anions which binds and removes calcium from the tooth surface (Featherstone, 2000).

It is said that dental erosion occurs when the tooth mineral is dissolved (Lussi, 2006). Following demineralisation enamel is able to recover and harden again if there has been no etching of the surface or tissue loss.

2.1.2 Aetiology of erosion

Many factors can lead to erosion which could be intrinsic, extrinsic, and idiopathic or a combination of these (Lussi and Ganss, 2014, Lussi and Jaeggi, 2008, Milosevic, 1998, Zero, 1996).

Intrinsic erosion is the result of teeth being exposed to gastric acids and such conditions occur in:

- Gastro-oesophageal reflux
- Medical conditions that cause self-induced or spontaneous vomiting (e.g. Morning sickness in pregnancy or Bulimia nervosa)
- Rumination disorder (disorder where food returns to the oral cavity after it was swallowed)

Extrinsic erosion is the result of external sources of acids such as:

- Dietary
- Environmental
- Medications
- Lifestyle

Idiopathic erosion is the result of contact with acids of unidentified origin where the patient history and their recollection was not capable of providing an aetiological explanation for the tooth wear. From the literature it seems that many clinical cases that report on enamel erosion due to idiopathic erosion are the result of a multifactorial aetiology that has not been clarified (Gupta et al., 2009).

In addition there are some predisposing factors that influence the development of erosive tooth wear (Lussi et al., 2003):

I. Chemical factors:

- pH, titrateable acidity and buffering capacity of the product
- Type of acid (pKa values)
- Adhesion of the product to the dental surface
- Chelating properties of the product
- Calcium concentration
- Phosphate concentration
- Fluoride concentration

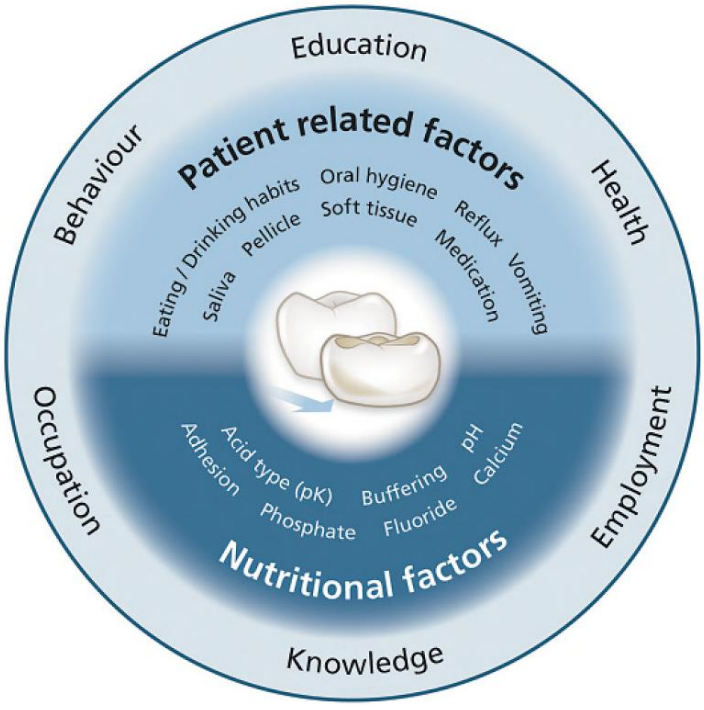
II. Behavioural factors

- Eating and drinking habits
- Healthier lifestyle: diets high in acidic fruits and vegetables
- Excessive consumption of acidic foods and drinks
- Night-time baby bottle feeding with acidic beverages
- Oral hygiene practices

III. Biological factors

- Saliva: flow rate, composition, buffering capacity, stimulation capacity
- Acquired pellicle: diffusion-limiting properties and thickness
- Tooth composition and structure (e.g. fluoride content as FHAP or CaF2-like particles)
- Dental anatomy and occlusion
- Anatomy of oral soft tissues in relationship to the teeth
- Physiological soft tissue movements

Figure 1 Relations of erosive aetiological factors (Lussi and Ganss, 2014)



2.1.3 Prevalence of dental erosion

Erosive tooth wear is difficult to assess due to different scoring systems between the examiners (Jaeggi and Lussi, 2014).

Erosion is a major problem in both adults and children. Studies over the years have presented the occurrence for dental erosion. Prevalence data from cross-sectional UK studies indicates that dental erosion increases between different age cohorts of young people over time. (Lussi and Ganss, 2014, Bardsley et al., 2004).

2.1.3.1 Prevalence in children 2-6 year old

Millward et al. (1994) investigated the erosion in 178 four year olds in Birmingham, UK and found almost half of them had signs of erosion with 17% of the lesions having progressed into dentine. In a study in Saudi Arabia Al-Malik et al. (2002) examined 987 children between the ages of 2-5 years and found signs of dental erosion in 31% with 17% of them having progressed into dentine.

Luo et al. (2005) conducted a study on 1949 children in China between the ages of 3-5 years who were examined and reported that 5.7% had erosion.

Murakami et al. (2011) reported that 51.6% of the Brazilian pre-schooler children had at least 1 tooth eroded and almost 93% of those lesions were in enamel. Dental erosion was related to good oral hygiene and high socio-economic levels with children presenting with more erosion (Mantonanaki et al., 2013).

Moimaz et al. (2013) found no association between erosion and gender, age, and tooth brushing habits. Table 1 summarises these studies and reports the prevalence.

Table 1 Prevalence of erosion in 2-6 year old children

Author	Country	Participants Age (years)	Sample size	Erosion Prevalence
Millward et al. (1994)	UK	4	178	50%
Al-Malik et al. (2002)	Saudi Arabia	2-5	987	31%
Luo et al. (2005)	China	3-5	1949	5.7%
Murakami et al. (2011)	Brazil	3-4	987	51.6%
Mantonanaki et al. (2013)	Greece	5	605	78.8%
Moimaz et al. (2013)	Brazil	4-6	1993	0.6%

2.1.3.2 Prevalence of erosion in children and adolescents

There are plenty of studies looking at the prevalence of erosive tooth wear in early and late mixed dentitions of which some are presented in Table 2. These studies mention areas that are more common to see tooth wear, like in the study of Milosevic et al. (1994) who reported that 305 children had lesions that involved dentine and those where the incisal edges of all incisors were involved. His findings were also confirmed by Al-Dlaigan et al. (2001) who found 51% of the 14 year old children had erosion into the dentine. They hypothesised that there was a correlation between low economic status and erosion incidence. Defects that involved dentine were usually of the edges of the mandibular and maxillary incisors. In 2001 Ganss et al. published a study that measured the erosion in orthodontic casts of 1000 children aged 8-14 years where erosive wear was 70% for the primary teeth and 11.6% for the permanent teeth. In his longitudinal examination there was an increase in the percentage of dental erosion in the permanent dentition. Van Rijkom et al. (2002) investigation was based on the study of Lussi et al. (1991) using he erosion index of Lussi and showed a smaller percentage in the younger age group. Similar data were presented in several epidemiological studies (Caglar et al., 2011, Arnadottir et al., 2010, El Aidi et al., 2010) that showed that more erosive lesions were detected in older children, males seemed to develop more erosion and that the occlusal surfaces of molars and the palatal surfaces of the upper incisors were the surfaces with the most severe lesions.

Table 2 Prevalence of erosion in children and adolescents

Author	Country	Participants Age (years)	Sample size	Erosion Prevalence
Milosevic et al. (1994)	UK	14	1035	30%
Al-Dlaigan et al. (2001)	UK	14	418	100%
Ganss et al. (2001)	Germany	8-14	1000	70.6%
Van Rijkom et al. (2002)	Netherlands	10-13 /15-16	345 / 400	3%-30%
Arnadottir et al. (2010)	Iceland	6-15	2251	15.7% - 30.7%
El Aidi et al. (2010)	Netherlands	10-12 / 13-16	622	30.4% - 44.2%
Caglar et al. (2011)	Turkey	7-14	83	52.6%

2.1.3.3 Prevalence of erosion in adults

Lussi et al. (1991) examined the severity of erosion in all tooth surfaces in two age groups. Erosion was detected in 3.5 teeth per person in the younger and 2.8 teeth per person in the older age group. The severity that was observed was around 29.9% for the younger and 42.6% for the older group, with the least being 3.6% for the palatal incisors in the younger group. Further it was shown that there was a significant association between acids from beverages and fruits with the presence of erosion (Mulic et al., 2012).

Table 3 summarises the changes in prevalence of dental erosion, how it was recorded from Lussi in 1991 and how it has been recorded in the last 5 years (2011-2016) in different countries.

Table 3 Prevalence of erosion in adults

Author	Country	Participants Age (years)	Sample size	Erosion Prevalence
Lussi et al. (1991)	Sweden	26-30 /46-50	391	3.6%-40%
Bartlett et al. (2011)	UK	18-30	1010	100%
Mulic et al. (2012)	Norway	18	1456	38%
Isaksson et al. (2013)	Sweden	20	494	75%
Bartlett et al. (2013)	European Countries	18-35	3187	26.5% -31.4%
Vered et al. (2014)	Israel	15-60	500	36.6% - 61.9%

2.2 Erosion prevention methods

Dental erosion is a product of acidic action on the surface of the teeth. Depending on the origin, the intensity and the person's susceptibility to acid, tooth surface loss becomes clinically evident when the condition persists for long periods. Understanding the aetiology of erosion can assist in its prevention and management.

Primary prevention entails patient information about the causes and the importance of individual preventive strategies. Also, depending on the percentage of erosion in a country population customised measures will need to be discussed. Secondary prevention comprises the prompt detection of the early stages of erosion as part of the customary dental examination and individually to arrange for management of the tooth surface loss.

Understanding the causes that lead to dental erosion helps in the treatment and prevention strategies. Usually restorative treatment is not necessary unless there are aesthetic of functional considerations.

In cases caused by extrinsic factors the therapy consists of changing the eating habits like adding calcium products to reduce the acidic effect of drinks or consuming fruit together with dairy products (Hughes et al., 2000). For cases due to intrinsic factors the therapy may be challenging due to the nature of the conditions like eating disorders. Medical treatment may be needed and the management of dental erosion may be with more symptomatic methods.

Symptomatic methods are the techniques that change the tooth surface so that the demineralisation due to acids is reduced. This includes substances that have acid-resistant properties and coat the surface of the tooth.

2.2.1 Fluoride for erosion protection

The effect of F on dental erosion has been investigated by Attin et al. (2003) who studied the effect of F added to citric acid on the erosion of bovine enamel under controlled conditions. The addition of calcium, phosphate or F to the citric acid solution resulted in significantly increased microhardness values compared with the controls. A similar but enhanced effect on microhardness was seen when all three were added together to citric acid (Amaechi et al., 1998). Amaechi and co-workers studied the effect of xylitol/fluoride combined on erosion of bovine enamel and found a significant difference in mineral loss in the pure orange juice group.

Fluoride is the cornerstone of prevention and remineralisation, and many methods of F application and supply to the tooth surface have been developed. The preventive effect of F is predominantly by its topical rather than its systemic effect. A constant supply of low levels of intra-oral F, particularly at the saliva/plaque/enamel interface, is of most benefit in preventing dental demineralisation and hence dental caries. Therefore, a treatment, which is able to raise intra-oral F, levels to a constant level, without the need for patient compliance would have a positive effect on improving oral health (Toumba and Curzon, 2005).

Fluoride has been used by patients and professionals in many different forms and concentrations. At home, patients usually are limited in the F concentrations and can use toothpastes and rinses with F concentrations under 1500 ppm. Professionals are not limited and can use liquids, gels and varnishes with higher concentrations of F up to 22,600 ppmF.

The frequently used F products such as toothpaste, rinses and varnishes in caries prevention made them valid fluoride products to be considered for the protection of dental erosion. On the market there are many products that advertise their protection properties against erosion. Studies testing this compound need to be interpreted and examined with care since the study design is variable and influenced by many parameters.

2.2.1.1 Toothpaste

Studies for toothpaste protection against erosion with 500 to 5000 ppm F concentrations and erosion/abrasion models reported protection of enamel between 0 and 26-46% for monovalent fluoride (amine fluoride, sodium monoflurophosphate and sodium fluoride) and around 55-67% for polyvalent metal cations (Ganss et al., 2011, Rochel et al., 2011, Moretto et al., 2010, Hooper et al., 2007). Both Moretto et al. (2010) and Rochel et al. (2011) in their studies the cycling regimes were for 7 days and used bovine tissue with soda drinks as the erosive medium. While Moretto used Sprite (pH 2.8) 4 times/ 5 min followed by 2 h remineralisation and the enamel blocks were exposed to one of the dentifrices. Rochels used Coca-Cola (pH 2.3) 4 times /2 min followed by 2h remineralisation and brushing 2 times daily.

Ganss et al. (2011) conducted two experiments for 10 days using human permanent teeth the slabs were exposed 6 times /2 min in citric acid (pH 2.6). In her first experiment she immersed the slabs in a slurry for 2 min and for the second study brushed for 15 sec during the 2 min slurry immersion period followed by 2 min immersion in the mouth rinse.

It is not clear whether brushing hinders the effect of F toothpaste or whether high concentrations of F offer more protection as these studies showed conflicting results.

Also studies with polyvalent metal cations seemed to have a better effect on the preventive surface loss. Overall the studies for toothpastes were relatively mild and of short duration. This indicates their suitability for prevention but they did not consider the primary dentition since the majority used permanent teeth.

2.2.1.2 Rinses

Solutions containing fluoride have been examined for their effect on the prevention of erosion with promising results. The majority of these studies were in situ and produced promising results with 18-19% reductions of surface loss (Ganss et al., 2010, Mathews et al., 2012). Monovalent fluoride seems to have less effect on erosion than polyvalent metal cations such as TiF_4 and SnF_2. TiF4 has a better effect at higher concentration than that available by professional applications (Hove et al., 2011). SnF_2 has been effective in reducing enamel erosion by 78-82% (Schlueter et al., 2011). Schlueter et al. (2011) ran the study for 7 days with 6 times / 5 min dipping in citric acid (pH 2.3) with concentrations of fluoride that were 250 ppm -1,900 ppm F.

The duration of the studies was short and they showed that the effect was related to the pH and F concentration (Levy et al., 2014).

2.2.1.3 Varnishes and Gels

Sorvari et al. (1994) was one of the first to use F varnish for the prevention of erosion with some effects. Murakami et al. (2009) used both primary (n=30) and permanent teeth (n=30) and divided them into three groups of APF gel (1.23 % F), NaF varnish (2.26 % F), and no treatment. They applied the gel for 4min and the varnish for 24h prior to the erosive challenge with 6 times /5 min immersion in a cola drink (pH 2.3) and 30 min in artificial saliva over 7 days. The results showed that prevention of primary enamel erosion by fluoride was not significant while permanent enamel showed a significant effect.

Fluoride varnishes and gels cannot be applied often and any effect is lost after a few days yet they are still valuable in preventing erosion.

2.3 Mechanism of action of fluoride slow-release devices

Many methods for slow F release have been attempted, including the development of F releasing amalgams (Fazzi and Vieira, 1977), fissure sealants (Cooley and McCourt, 1990), composite resins, compomers, and glass-ionomer cements (Karantakis et al., 2000). Controlled and sustained F delivery systems can deliver F to the tooth surface for caries prevention with minimal patient compliance. These systems are similar to those used for birth control, treatment of glaucoma and for motion sickness, and can be considered a method of controlling dental caries in high-risk patients. Therefore, slow-release topical F devices were tested in vitro, in situ, and in vivo. These methods of F delivery have been shown to exhibit a burst effect, in which larger amounts of F are released on the first and second days. Further it is claimed that these materials have the ability to recharge (Toumba, 2001).

Types of fluoride slow-release devices:

- Copolymer membrane, (developed in the United States).
- Hydroxyapatite-Eudragit RS100
- Bioadhesive fluoride tablets
- Glass bead, (developed in Leeds, United Kingdom) (FGSRD)

2.3.1 Copolymer membrane

This membrane – controlled reservoir was developed by Cowsar et al. (1976). An acrylic polymer membrane encapsulates granules of sodium fluoride (NaF). The F release rate is controlled by 30/70 HEMA/MMA copolymer membrane when it becomes hydrated, small quantities of granulated NaF are diluted and they are reliably released according to Fick's first law.

Billings et al. (1998) studies a copolymer device that was 8 mm in length, 3mm in width, and 2 mm in thickness. The copolymer devices come in two sizes for molars and premolars and usually are attached to the buccal surfaces of first permanent molars by means of stainless steel retainers, standard orthodontic bands or are bonded to the tooth surface by adhesive resin (Mirth et al., 1982). Copolymer devices were reported to release 0.02 mgF/ day to 1.0 mgF/day for up to 180 days (Billings et al., 1998, Mirth et al., 1983, Mirth et al., 1982).

2.3.2 Hydroxyapatite-Eudragit RS100

Altinova et al. (2005) prepared and tested hydroxyapatite tablets. Eudragit RS100 diffusion-controlled F-system can release 0.15 mg F/day for one month. The tablets, each containing 18 mg of sodium fluoride and were attached to the buccal surfaces of the first maxillary molar teeth and were prepared to have one concave and one flat surface with a diameter of 5 mm, a thickness of 2 mm and weighing 70 mg.

2.3.3 Bioadhesive fluoride tablets

The bio-adhesive characteristics of F releasing tablets for oral use were made from modified starch, polyacrylic acid (PAA), polyethylene glycol (PEG) and sodium carboxymethylcellulose (CMC) (Bottenberg et al., 1991). Modified maize starch tablets containing 5% (w/w) PAA and PEG with a M.Wt of 300,000 daltons proved to be the most suitable formulation for a fluoride-slow-release tablet with bio-adhesive properties. In-vitro, the tablets released all of the fluoride within an 8 h period, with a high initial release of F (Bottenberg et al., 2000).

2.3.4 Fluoride glass slow-release devices

The fluoride glass slow-release devices (FGSRD) were developed at Leeds University in1984. Their function has been tested over the years with in vitro, in vivo, and in situ studies in animals and humans.

FGSRD's were tested with concentrations of 13.3%F, 18.3%F, and 21.9%F and it was concluded that the 13.3%F produced a higher F concentration in saliva. Andreadis examined the relative solubility of these FGSRD's and found that the devices released more F when the environment was acidic (Andreadis et al., 2006, Toumba, 1996). Thus more fluoride is released exactly at the time that it is needed most and thus these devices can be considered as "smart" devices.

The duration of release of F and the concentration in saliva was studied by Bashir, 1988 reporting that 19%F and 13%F released a maximum of 0.03 to 0.04 ppm F for a prolonged period of 18 months.

Toumba in a randomised double blind clinical trial with high caries risk children examined the effects of FGSRD for the prevention of dental caries. This study reported 67% fewer new carious teeth and 76% fewer new carious surfaces (Toumba and Curzon, 2005, Toumba, 2001). FGSRD's are the only slow release devises that have been critically appraised by a Cochrane review by Bonner et al., 2006 .

Extensive studies on the FGSRD effects on the alleviation of dentine hypersensitivity (Malik-Kotru, 2009), plaque (Abudiak, 2007) and orthodontic demineralisations (Tatsi, 2014)), have all been researched with promising results observed.

A research study (Malik-Kotru, 2009) in vitro using the FGRD's showed occlusion of dentinal tubules with scanning electron microscopy (SEM). EDAX scans using SEM of the material occluding the dentinal tubules gave a calciumphosphate ratio of 1.76 which is identical to that of apatite. Malik-Kotru also conducted a double blind randomised controlled clinical study of the FGSRD's (F test devices and placebo non-F devices). Both groups showed a significant alleviation of dentinal hypersensitivity with no significant differences between the test and placebo groups.

The fluoride levels in plaque biofilms and saliva were measured over a period of 7 days in 65 subjects using the FGSRD's and placebo devices. No differences between the study groups were observed and longer periods of plaque biofilm collection were advised (Abudiak et al. 2011).

Test and placebo FGSRD's were tested for their effect in the prevention of white spot lesions (WSL) in an orthodontic study of 70 subjects undergoing fixed appliance therapy. The FGSRD's were shown to prevent WSL's but more studies with increased numbers of participants were recommended (Tatsi, 2014).

2.3.4.1 Shape of FGSRD

The original FGSRD had a low retention rate, and therefore a new-shaped device was developed. This device was kidney shaped, 6mm long, 2.5mm high and 2mm thick. One surface was concave, and this was attached to the buccal surface of the tooth. The opposite surface was convex, exposing a larger surface area to the oral environment. All around the remaining surface, a groove was placed, to enhance retention with the composite material used for attachment to the tooth surfaces (Andreadis et al., 2006). In 2006 the device was shaped in the form of a disk that is placed within plastic brackets to help with the attachment and future replacement of the bead.

The device is usually attached to the buccal surface of the maxillary first molar using adhesive resins (Toumba, 2001).

The glass devices have different solubility rates depending amongst other factors on the oral pH. In comparison to the copolymer membrane device, the glass devices have been shown to have a longer lifetime releasing F continuously for up to 2 years (Toumba and Curzon, 2005, Bashir, 1988).

2.3.4.2 Toxicity of FGSRD

Curzon and Toumba investigated the ingestion of the FGSRD in human subjects in which no changes were noticed. Therefore, if a FGSRD becomes de-bonded and swallowed, there is no risk of absorption of F into the blood stream (Curzon and Toumba, 2004). When FGSRDs are swallowed the devices either pass through very quickly and they do not release F capable causing problems (Toumba, 2001).

Animal studies have also demonstrated that no toxic effects were observed in dogs, after ingestion of the copolymer devices containing a six month supply of F (Mirth, 1979).

2.4 Model systems used in the study of dental erosion

2.4.1 In vitro models

In vitro studies on dental erosion have tried to create erosive lesions on enamel by using different techniques. In most of these in vitro studies, erosive lesions are created by simply immersing a tooth into the erosive challenge like citric acid or soft drinks like orange juice for a period of time. This method provided information on the erosive potential of these products, however it increased the erosive effects due to the absence of factors present in the oral environment such as saliva remineralisation, salivary pellicle and buffering capacity of saliva (Eisenburger et al., 2001, Hunter et al., 2000, Lussi et al., 1995).

Amaechi et al., (1999) used a modified technique to create dental erosion lesions. They immersed teeth in stirred pure orange juice at regular time intervals six times per day for 5 minutes on each occasion for a period of 24 days, giving 30 minutes of daily exposure or a total of 12 hours of exposure to orange juice. The immersion was carried out at room temperature. In between the exposures the teeth were either stored in artificial saliva or in de-ionised distilled water. These groups were compared with prolonged exposure for 12 hours to a third group in pure orange juice. Mineral loss of the groups was measured using microradiography and it was found to be significantly lower in those specimens cycled in orange juice and artificial saliva compared with those cycled in orange juice and de-ionised distilled water and those from the single 12 hours immersion in orange juice.

It was concluded that the modification technique for creation of dental erosive lesions using artificial saliva had reduced the potential erosive effect of orange juice. Our aim was to use a methodology to study dental erosion in vitro in a situation close to the real life scenario. Therefore, a modification of the Amaechi et al. (1999) technique was employed that was previously used at Leeds University in previous dental erosion studies (Abdullah, 2009).

2.4.2 In situ models

In situ models involve the use of appliances or other devices that help in the recreation of natural oral conditions (Zero, 1995). In studies for demineralisation in vital teeth, devices such as small gold cups were used for the first time (Bunting et al., 1926) and gold plates (Nygaard Östby et al., 1958). Koulourides and Volker in 1964 used in situ models in order to check the carcinogenicity of foods and topical materials with cariostatic effect. Since then Koulourides modified and used his first model in different studies. In situ models continue to be used in dental research as they act as a transitional step from in vitro studies to clinical trials (Clasen and Øgaard, 1999, Manning and Edgar, 1992).

The advantages of this model are that the experiment is conducted in the human oral environment instead of laboratory conditions where the condition are somewhat standardised and not uncontrollable like in vivo models which have too many variables. The disadvantages of the in situ models are that they usually have a small number of participants and there is a dispute over the applicability to the general population. Also, they are very dependent on participant compliance.

2.5 Dental erosion evaluation techniques

Many techniques have been used in order to investigate the loss of tooth structure during erosion (Barbour and Rees, 2004). The most well-known of these are:

- Micro-indentation
- Surface Profilometry
- Microradiography
- Chemical analysis
- Scanning electron microscope (SEM)

Profilometry and micro-radiography are readily applicable to enamel erosion at more advanced stages, but to investigate the earlier stages of erosion it is preferable to use more sensitive techniques such as micro-indentation. More sensitive still is nano-indentation, which is likely to find increasing application in the field (Barbour and Rees, 2004).

The study design, the study model, and the methods usually determine the test that is chosen. Several techniques have been discussed with respect to their application to enamel erosion studies.

2.5.1 Microhardness

Microhardness indentation measures the resistance of the tooth surface to a penetration force. It is a function test of the amount of porosity of the superficial enamel layer that can show mineral changes in tooth surface lesions (Koulourides, 1971).

Indentations are created in the tooth surface with a Vickers or a Knoop diamond. The diamond is positioned on the sample with a calculated load for a specific duration after which the indentation lengths are then measured microscopically in µm (Ten Bosch and Angmar-Månsson, 1991).

There are two types of microhardness tests cross-sectional microhardness (CSMH) and surface microhardness (SMH). In CSMH the load is applied parallel to the tissue anatomical surface while in SMH the load is applied perpendicular. SMH when is used can give qualitative information on the mineral of the surfaces (Arends and Ten Bosch, 1992, Arends et al., 1980). Microhardess can be used to evaluate the hardness of the tooth structure when there is a direct indent of the length of the reading which changes depending on the elasticity (Hosoya et al., 2000).

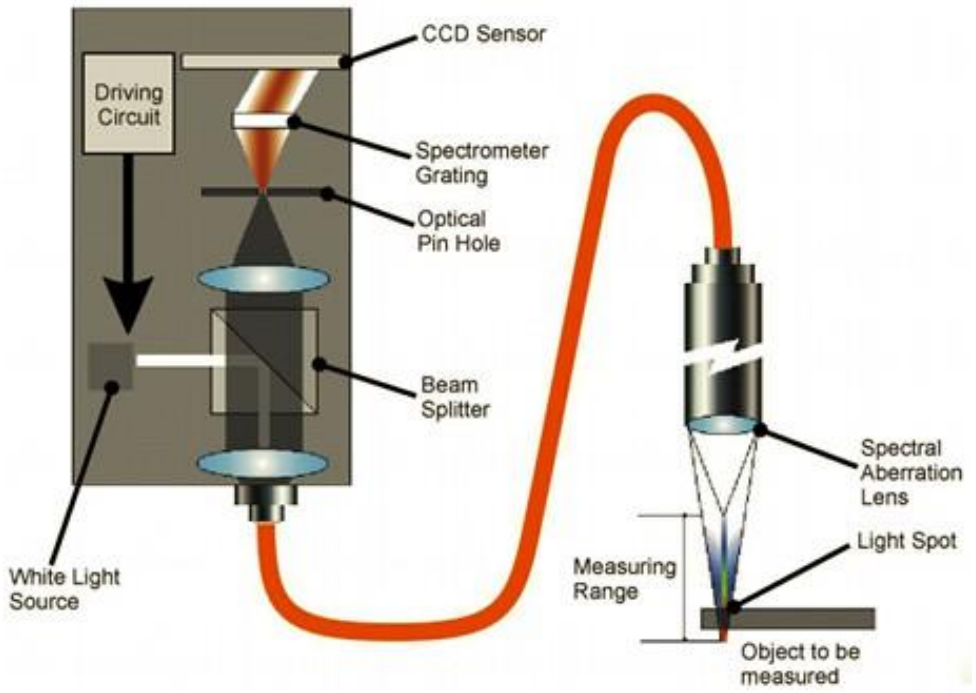
2.5.2 Surface profilometry

Surface profilometry has been one of the common laboratory techniques for assessing tooth surface loss. The results of the enamel surface are accurate, highly reproducible, simple and fast to obtain. However, the enamel slabs have to be flat prior to the experiment (Barbour and Rees, 2004).

The surface profilometer used in this study has got the advantage that there is no direct physical contact with the assessed surface during the scan and no danger of scratching the eroded/abraded area. It provides accurate measurements even when the slabs are positioned at an angle since they can be levelled in the horizontal axis. Therefore the measurements are reproducible and accurate. Each scanning takes just a few minutes and the data can be saved easily for analysing at any time.

Using a surface profilometer loss of dental hard tissue can be determined by scanning specimens with a laser. In Figure 2 there is a schematic description of the basic operational principles of the optical surface profilometer.
This method of analysis has been tested before to check the erosive potential of products in vitro such as acid solutions (Hughes et al., 2000), mouth rinses (Pontefract et al., 2001), herbal teas (Phelan and Rees, 2003) toothpastes and CPP-ACP products (Rees et al., 2007).

Figure 2 Schematic description of the basic operational principles of the surface profilometer



The laser stylus may produce sharp edges at the bottom of the surface which result in artefacts and the same can happen in the enamel due to acid attack which leads to surface roughening of about 0.4m. So, reliable detection losses below 1µm are generally difficult to measure with profilometry. Hooper et al. (2003) demonstrated that profilometry was able to distinguish between different abrasivities of toothpastes creating a hard tissue loss of about 0.5µm. Barbour and Rees (2004) observed that if the surface was flat and polished then the detection of surface loss could be even less than 1µm.

In studies using profilometry, parts of the surface are protected by nail varnish or adhesive tape prior to the erosive or abrasive challenge so reference areas exist to allow comparison between the levels of the untreated and treated surfaces.

2.6 Aim

The aim of this project was to investigate the effect of fluoride glass slow-release devices on the prevention of dental erosion in human primary and permanent dental enamel in vitro.

2.7 Null hypotheses

Hypothesis 1: There is no difference between test and placebo glass slow-release devices on the prevention of an erosive challenge on the dental enamel of permanent teeth.

Hypothesis 2: There is no difference between test and placebo glass slow-release devices on the prevention of an erosive challenge on the dental enamel of primary teeth.

Hypothesis 3: There is no difference in the prevention properties of the FGSRD between the permanent and primary teeth.

3 Materials and Methods

3.1 Ethical approval

This study was conducted in the bioengineering dental laboratory at Leeds School of Dentistry. Permanent and primary human teeth were requested from the Leeds School of Dentistry tissue bank for which a less extensive version of the work protocol was submitted with an application form (Appendix 1). This application was approved (Appendix 2) and permanent and primary teeth were then collected from the tissue bank (Appendices 3 and 4).

3.2 Materials

3.2.1 Equipment

- Well Diamond Wire Saw (Well Walter EBNER, CH-2400 LeLoche)
- Techne Dry-Block for wax melting
- Grinding Machine Veneer Grinder
- Surface profilometer (Scantron ProScan 2000, version 2.1.1.8, Scantron Industrial Products Limited, Somerset, England)
- Duramin Indenter Machine (Struers A/S, DK 26-10, Denmark)
- Water Purelab Option-S
- Incubator (Gallenkamp)
- F meter Metrohm 781 pH/ions Swiss made
- pH meter Orion Model 900A
- Magnetic stirrer
- Balance (Max. 210mg) (HM-200,A&D Instruments Ltd. Abingdon, UK)
- Brushing machine
- Timers

3.2.3 Materials

- Enamel slabs from human permanent and primary teeth. Consent was taken from tissue bank (Appendices 3 and 4)
- FGSRD 13.3% (Ultradent Inc., South Jordan, Salt Lake City, Utah, USA)
- Placebo devices without fluoride made by the same manufacturer.
- Impression Compound (Kerr green wax)
- Mounting wax…
- Yellow wax...
- 600,1000,1200 -grade fine grit abrasive paper (3M)
- Silicone mould compound silastic S
- Cold resin Stycast 1266
- Perspex plastic holders
- Nail varnish (passion red colour, MaxFactor, England, UK)
- Fluoride-free toothpaste (Boots company)
- Medium toothbrushes (Basic, Sainsbury's company)
- Light cured composite resin (Spectrum, DENTSPLY, DeTrey, Germany)
- Container for dipping and storing solutions
- Artificial saliva chemicals (for night and day saliva)(Tables 4 and 5)
- Citric acid monohydrate, Analar NormaPur VWR
- Distilled water

3.3 Methods

3.3.1 Sample size calculation

Sample size was determined based on the objective of the study: to investigate in vitro the efficacy of fluoride glass slow release devices for the prevention of dental erosion of human dental enamel in vitro over 28 days. Assuming a minimum difference as significant is enamel loss of 5 µm, Standard deviation (SD) is for primary teeth 2.3 µm and for permanent teeth 3.5 µm from a study at University of Leeds (Malinowski et al., 2014), power calculation is 99%, significance level 1%. Sample size formula was used.

(http://www.stat.ubc.ca/~rollin/stats/ssize/n2.html)

mu1 (mean of population 1), mu2 (mean of population 2), and sigma (common standard deviation) α (type I error rate) and the power.

Primary:
Enter a value for mu1: 6.657
Enter a value for mu2: 11.499
Enter a value for sigma: 2.289
Enter a value for α (default is .05): 0.01
Enter a value for desired power (default is .80): 0.99
The sample size (for each sample separately) is: **10**
Permanent:
Enter a value for mu1: 8.224
Enter a value for mu2: 15.418
Enter a value for sigma: 3.549
Enter a value for α (default is .05): 0.01
Enter a value for desired power (default is .80): 0.99
The sample size (for each sample separately) is: **11**

The previous formula recommended 10 samples per group for primary and 11 samples per group for permanent dental enamel slabs. This sample size determination took into account that data are clustered within a slab or they are repeated measurements. Repeated measurements are not independent. Traditional statistical methods require observations to be independent. Therefore, the sample size was inflated by the design effect (DEFF).

DEFF= 1+(n-1) x ICC, where (ICC) is intra-class correlation of repeated measurements from previous study, (n) is the number of repeated measurements per slab.

Inflating sample size by

DEFF=1+(n-1) x ICC

DEFF= 1+(3-1) x 0.3= 1.6

Inflating sample size 10x1.6=16 slabs per group for primary

Inflating sample size 11x1.6=17.6 (18) slabs per group for permanent

To allow for loss of samples it was decided that a minimum of 20 samples per group was to be used.

Finally in the experiment 20 slabs per group for primary and 24 slabs per group for permanent were included.

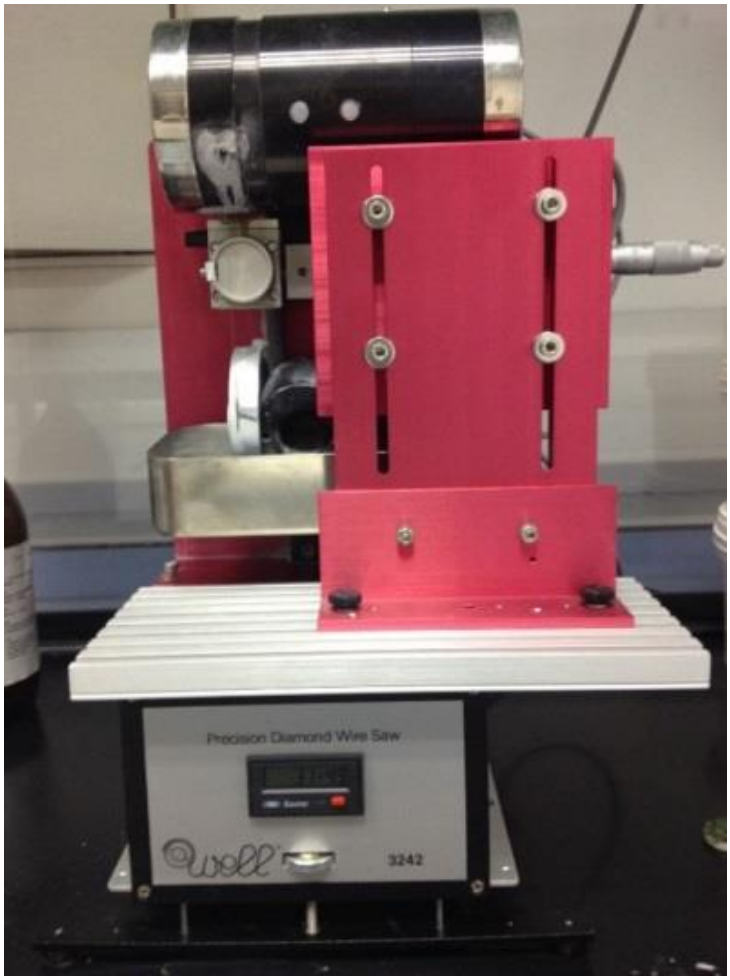
3.3.2 Enamel slab preparation

Human permanent molars and premolars and primary molars extracted for various reasons and stored in a solution of distilled water and 0.1% thymol were used.

Before sectioning, the teeth were cleaned using a spoon excavator and a toothbrush with pumice powder and stone to remove any soft tissue. In addition, they were carefully checked for cracks, caries, or other malformations.

The cleaned teeth were dried and attached whole with green wax (greenstick) on plates that fitted into the cutting machine " The Well Diamond Wire Saw, water-cooled, cutting Machine" (Figure 3).

Figure 3 Well Diamond Wire Saw



Each tooth was de-coronated and then sectioned so buccal and palatal / lingual surfaces were obtained where enamel was thicker. After this they were carefully sectioned again to form the slabs (Figure 4).

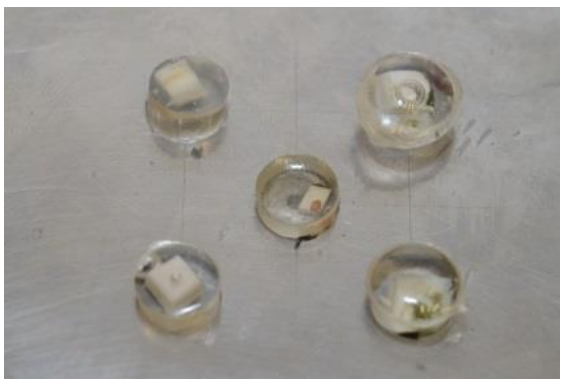
Figure 4 Teeth after de-coronation and sectioning to form the final slabs

The slabs were placed in silicone moulds and then embedded in clear resin (Stycast 1266) and left for 24 hours to dry in order to form circular resin blocks of 3 mm thickness and 7.5 mm width (Figure 5).

Figure 5 Silicone moulds with the enamel slabs

To ensure flatness of their surfaces the blocks were placed in rectangular steel blocks, which had circular holes of 3 mm depth. 600-grade fine grit abrasive paper followed by 1200 and 2000 grade were used respectively to grind the enamel surfaces after mounting in resin to the same thickness as the holes in the steel blocks. A grinding machine was used for that purpose (Figure 4). The slabs were then cleaned and care was taken not to remove the enamel layer.

Figure 6 Resin blocks through the grinding process



3.3.3 Selection of slabs

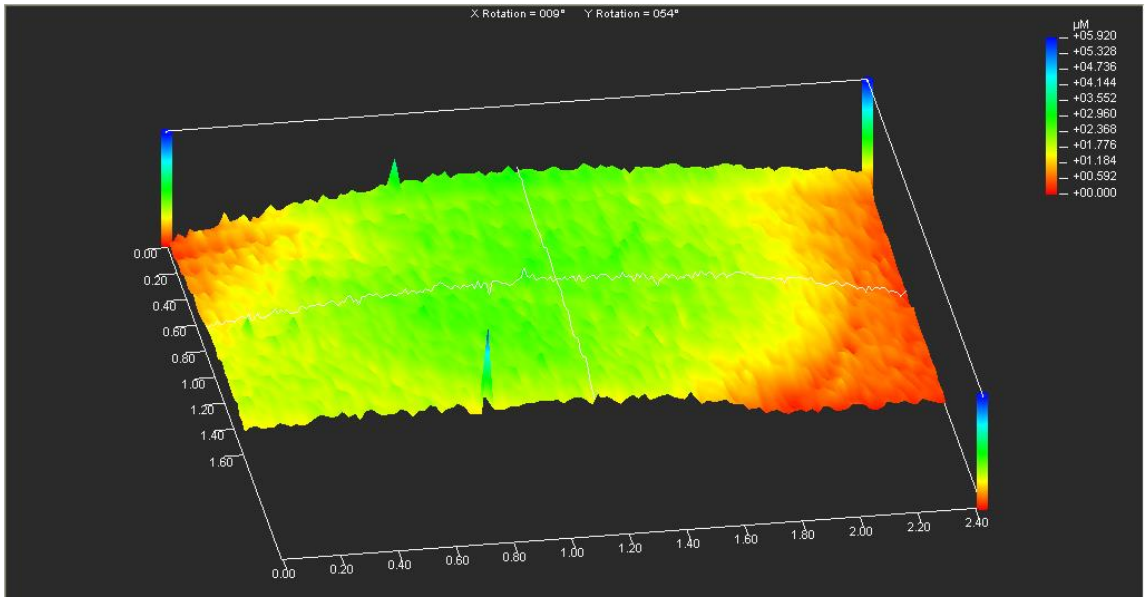
After grinding the slabs went through tests to make sure that they were flat and the exposed dental tissue was that of enamel. These tests were:

3.3.3.1 Surface profilometry

Baseline measurements of the surface profile of the slabs were assessed using a surface profilometer (Scantron ProScan 2000) to ensure that the average depth range was (mean ISO Rz) less than 1.5 µm. The measurement was achieved by placing the sample on the key stage of the Scantron ProScan and using a 150 mm height of the sensor as standard. Sample rate was set at 300Hz and the sensor that was used was S5/03. The step size used was 0.01 mm. After scanning the reading was auto levelled, function interpolate x4 and warpage 1 to remove spikes from dust. A profile analysis where mean ISO Rz was measured (Figure 7) if the measurements for mean X and Y was <1.5 the slab was sent for microhardness testing.

Figure 7 Flat surface Profile Analysis mean ISO Rz< 1.5 µm

Calculation	Mean X	Min X	Max X	Count X	Mean Y	Min Y	Max Y	Count Y	Units
ISO Am	+05.046	+03.963	+05.601	201	+05.043	+03.698	+05.773	41	µm
ISO Ra	+00.757	+00.377	+01.247	201	+00.789	+00.493	+01.091	41	µm
ISO Rz	+00.897	-	+02.223	201	+01.134	-	+02.502	41	µm
ISO Rmax	+01.624	-	+05.972	201	+01.814	-	+05.200	41	µm
ISO Rp	+01.061	+00.543	+02.000	201	+01.400	+00.841	+03.091	41	µm
ISO Rq	+00.686	-	+01.487	201	+00.830	-	+01.411	41	µm
ISO S	+08.293	-	+380.000	201	-	-	-	41	µm
ISO Wt	+03.643	+01.807	+06.555	201	+03.232	+01.735	+06.626	41	µm

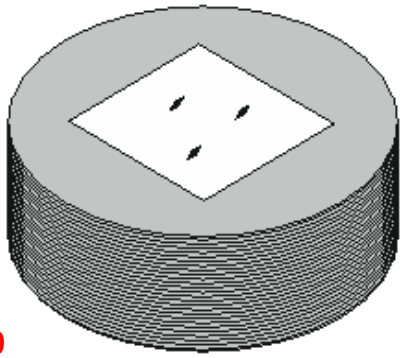


3.3.3.2 Microhardness

Baseline measurements were recorded using Knoop microhardness. Microhardness was assessed using a computer-aided Duramin Indenter Machine (Struers A/S, DK 26-10, Denmark). The indentations were made using a Knoop diamond under a 100 g load for 30 seconds. The length of indenter penetration was measured by means of an image analysis system. Three indentations, spaced more than 50 μm apart were taken in order to make sure that the visible tissue was enamel. The length of each indent was recorded. An average 64 ±3 μm was needed in order for the slabs to be included in the study (Figure 8).

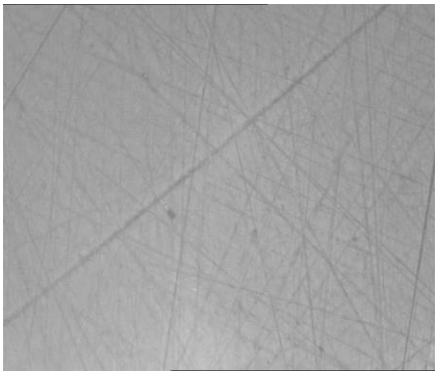
Figure 8 a) Indentation machine, b) Diagram of the reading, c) Dental tissue as seen in microscope, d) Inclusion reading, e) Exclusion reading

a



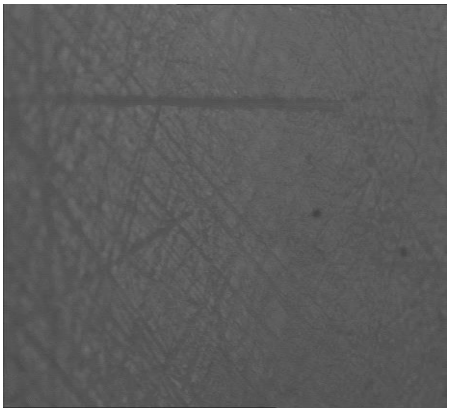
b

c



d

e



3.3.4 Blindness and Randomisation

The glass slow release devices were randomised into two groups. Test and placebo groups were coded (by the lead supervisor) as A and B and the blindness was maintained until the end of the experiment. Enamel slabs were randomly allocated to each study group. For the slabs randomisation (https://www.randomizer.org/) was used. We generated 2 sets (primary, permanent enamel slabs) for 48 slabs (24 slabs for each group) were 0 and 1 was for division in the two groups.

Set 1

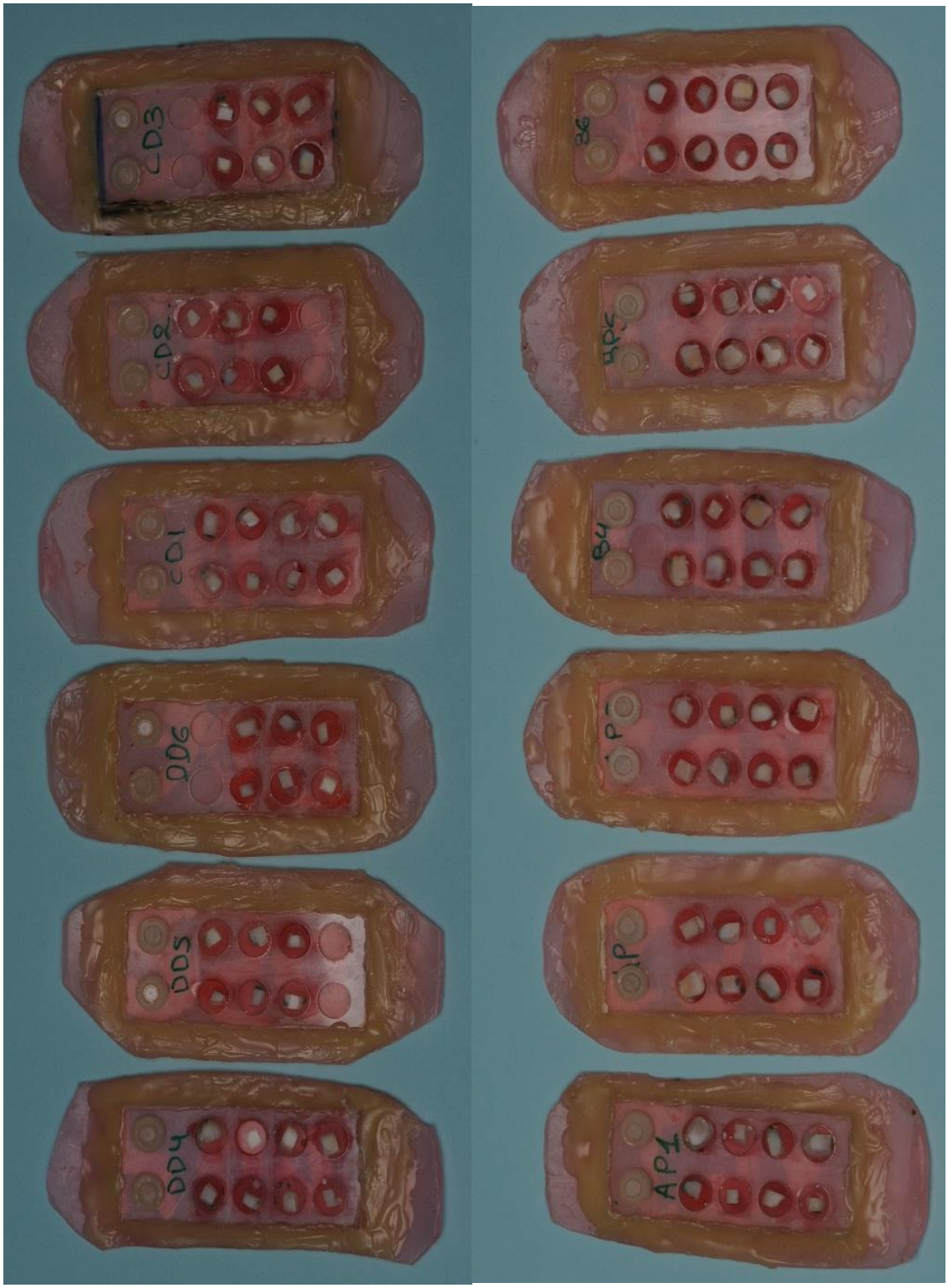
1, 1, 1, 1, 0, 0, 1, 1, 0, 1, 0, 0, 1, 0, 0, 1, 1, 0, 0, 1, 1, 1, 0, 0, 0, 1, 1, 1, 1, 1, 1, 0, 1, 1, 0, 0, 1, 0, 1, 0, 1, 0, 1, 0, 1, 1, 0, 0

Set 2

1, 0, 0, 1, 0, 1, 0, 1, 1, 0, 0, 1, 1, 1, 0, 0, 0, 0, 0, 0, 1, 1, 1, 0, 1, 0, 1, 0, 0, 1, 0, 0, 0, 0, 1, 1, 0, 1, 0, 0, 1, 0, 0, 0, 1, 1, 1, 0

Where Set #1 and Set #2 were for primary and permanent teeth with 0 and 1 the groups with either fluoride or non-fluoride glass slow release devices depending on the coding allocation (Figure 9). When the slabs were analysed, the investigator did not know to which group the enamel slab belonged, making the analysis completely blind.

Figure 9 Sets 1 and 2 of the human enamel slabs



3.3.4.1 Groups**:**

After, the slabs were cleaned with de-ionised distilled water and methanol placed in the plastic trays (Figure 10) and then covered with nail varnish (red colour, MaxFactor, England, UK) except for a small window that was left exposed and these were divided in Groups (Figure 11).

Group A: permanent enamel slabs with non-fluoride glass slow release devices.

Group B: permanent enamel slabs with fluoride glass slow release devices.

Group C: primary enamel slabs with non-fluoride glass slow release devices.

Group D: primary enamel slabs with fluoride glass slow release devices.

Figure 10 The slabs within resin blocks and held in a special holder created to hold each test group

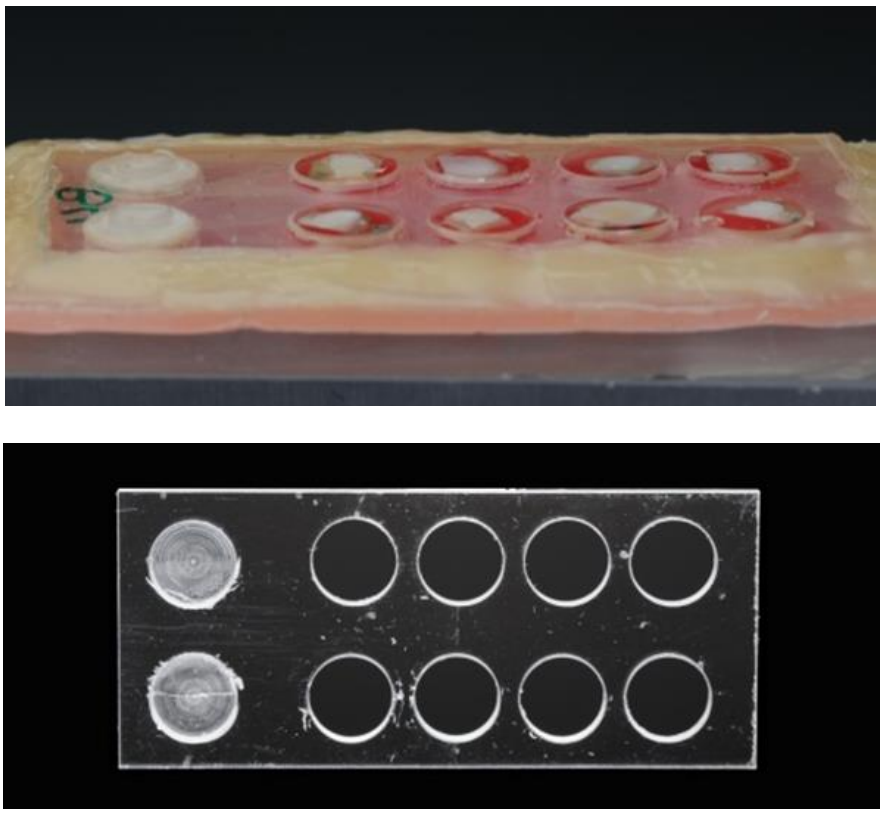
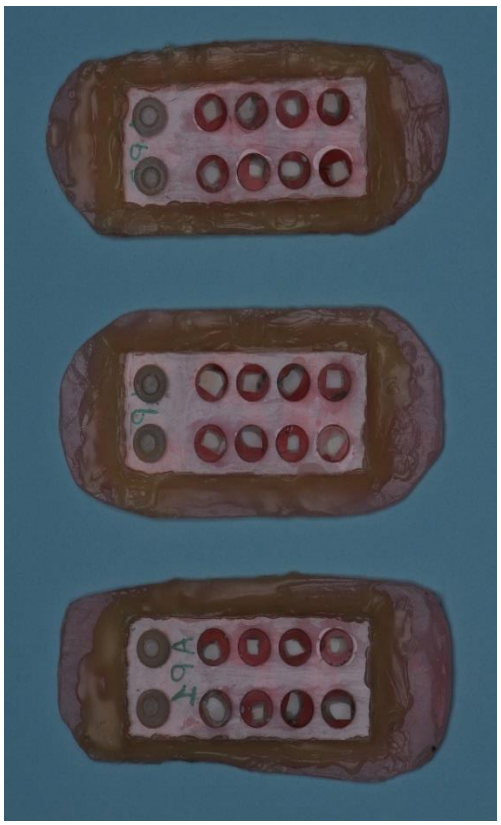
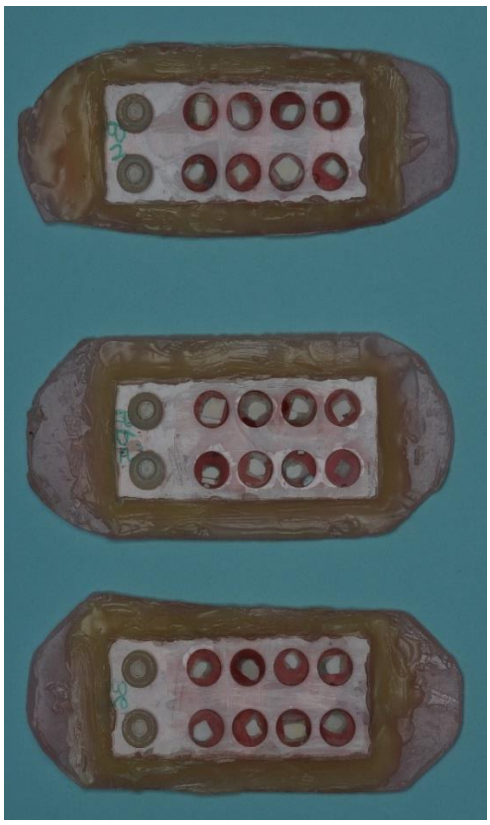
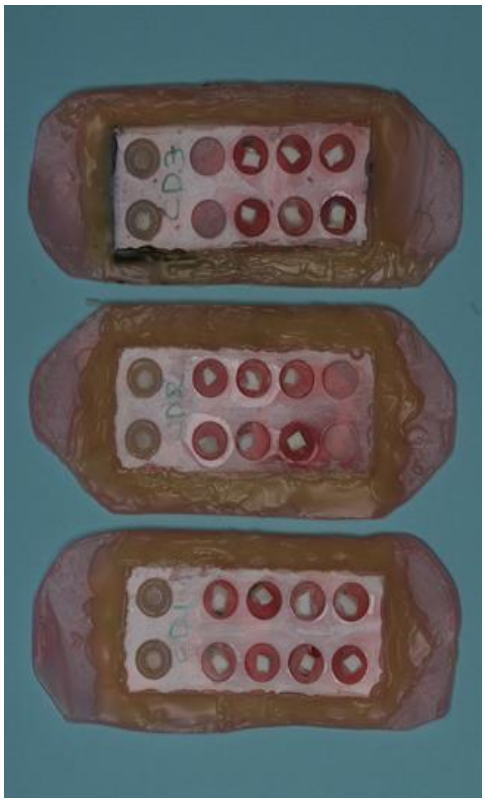
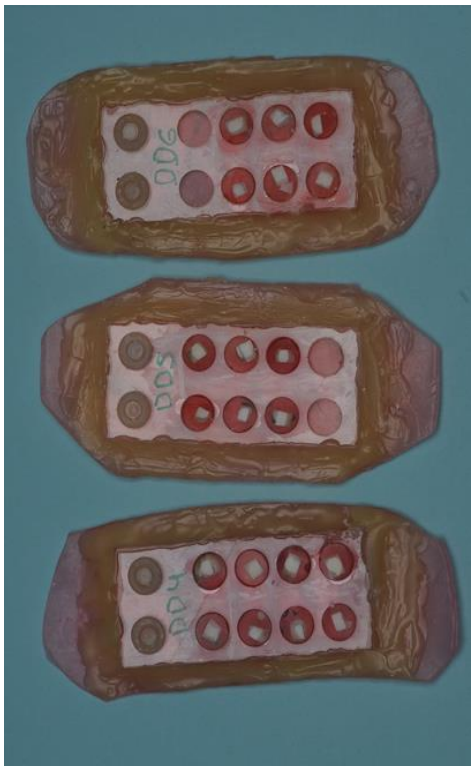


Figure 11 Groups of enamel slabs after randomisation

Group A and B		
Group C and D		

3.3.5 Experimental protocol/regime

A special tray with 2 blind holes and 8 holes in the resin blocks was used to hold the blocks (Figure 10). Resin blocks were secured in position using adhesive wax. The slabs were immersed in a solution for two minutes five times daily in 0.3 % citric acid (pH 2.6) for a period of 28 days (Figure 13). Citric acid was prepared by adding three grams of mono-hydrate citric acid to one litre of de-ionised distilled water. Each group of slabs was immersed at room temperature in fresh 200 ml aliquots of citric acid each time. On each occasion, before immersion in citric acid, the slabs were taken out of the artificial saliva. The slabs were also rinsed in de-ionised distilled water (pH 6.85±0.05) after treatment before they were returned to the artificial saliva, which was changed twice daily (Figure 14). Two artificial saliva solutions were used in this study. The first solution was used for day time during the pH cycling, between the acid exposures. The second solution was used to store the slabs during the night. The day saliva was a supersaturated solution that allowed remineralisation of enamel slabs, the night saliva was a saturated solution that maintained the enamel condition and did not provide any minerals exchange.

The artificial saliva composition was based on the electrolyte composition of natural saliva and it was advised to be used in order to eliminate any precipitation on the enamel surface (as provided by Dr RP Shellis, Department of Oral and Dental Science, University of Bristol, Bristol, UK). Day time and night time saliva were prepared as shown in Table 4 and 5 respectively.

Table 4 Day time artificial saliva

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

To 5 L distilled water the above components were stirred until all had dissolved The pH was adjusted to 6.8 by adding NaOH and HCL solutions. The solution was kept at room temperature and used within 2 days.

Table 5 Night time artificial saliva

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

The night-time saliva was made up using the same procedure as above.

Between immersions in citric acid the slabs were left immersed in artificial saliva for 60 minutes to enable remineralisation. The slabs were kept in an incubator at 37.0^{o}C at all times except while they will be being immersed in citric acid (Figure 12). Artificial saliva was changed twice daily to prevent any contamination or bacterial growth. A 60-minute gap was left between day time erosive challenges and between dipping's in toothpastes and the erosive challenges. After the dipping in the erosive solutions the slabs were rinsed with de-ionised water.

Figure 12 Incubator at 37.0^{o}C

During the cycling period, the slabs were analysed with the surface profilometer to measure the amount of surface loss at days 14 and 28. Concurrently the amount of F released from the FGSRD was measured.

Figure 13 Flow chart for all Groups

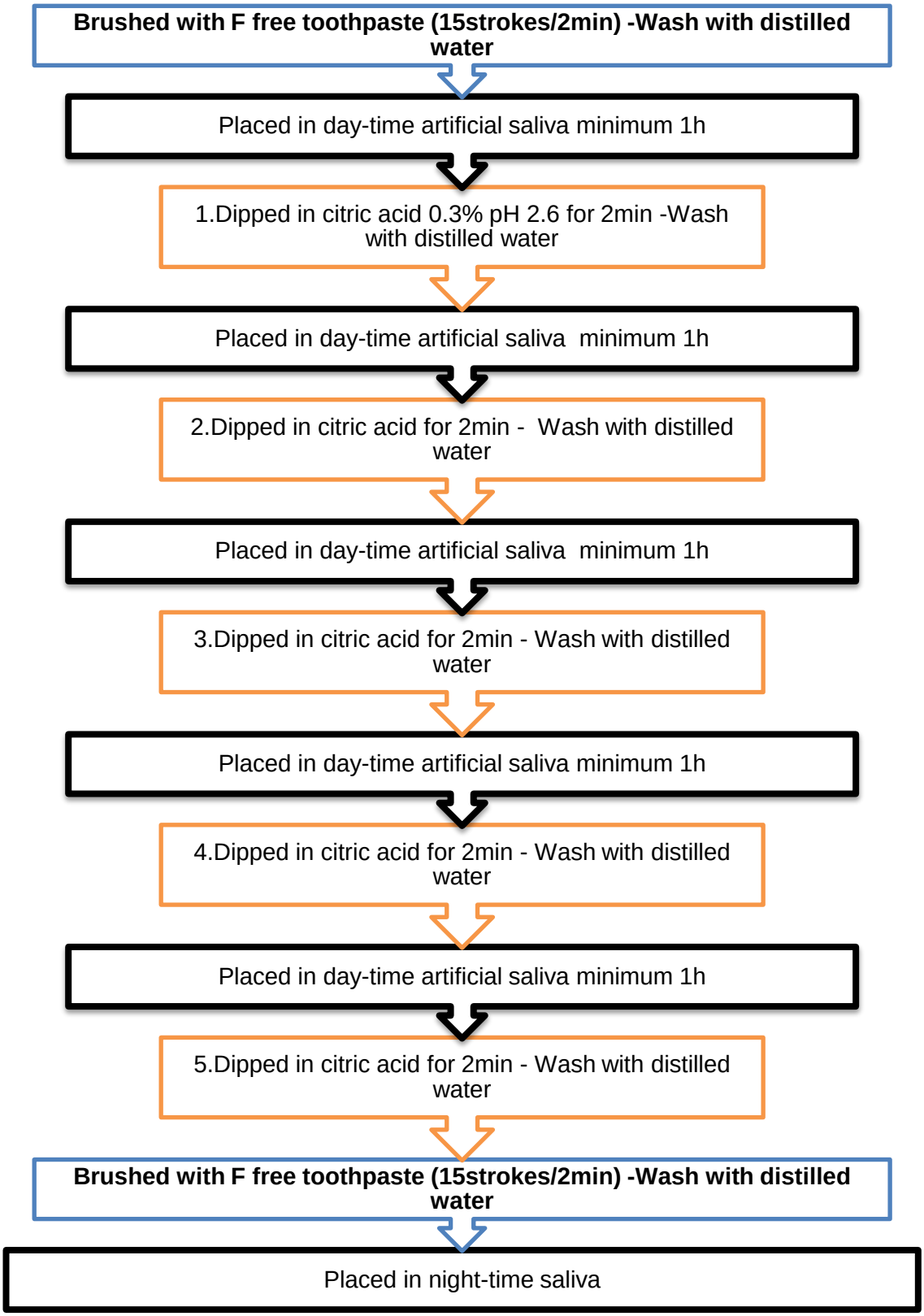
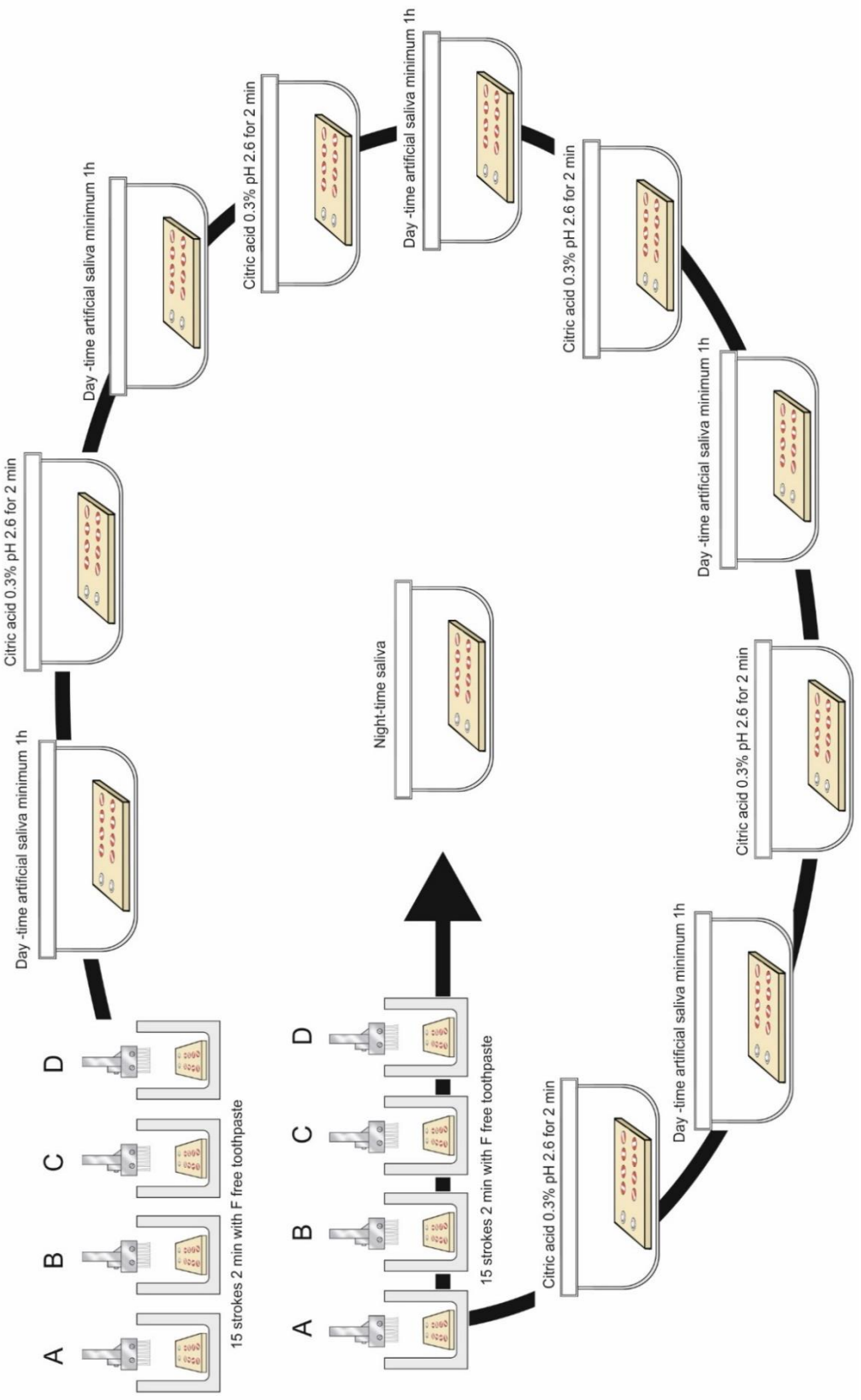


Figure 14 Illustration showing the cycling technique used for 1 day. After placement in artificial saliva the containers were transferred in an incubator for a minimum of 1h



3.3.6 Data collection

At the end of the cycling period at 14 and 28 days, the slabs were rinsed with de-ionised distilled water and air-dried. The nail varnish was then removed using acetone and the enamel surface was cleaned with ethanol to ensure that all residues will be removed. The slabs were then kept in de-ionised distilled water and the FGSRD were placed in centrifuge tubes and left at room temperature.

The slabs were scanned with the profilometer that was set up using the same parameters as for the baseline measurements. The sample was placed on the key stage of the Scantron ProScan and using a 150 mm height of the sensor as standard. The sample rate was set at 300Hz and the sensor that was used was S5/03. The step size used was 0.01 mm. After scanning the reading was levelled in three points A, B, and C (Figure 15) function interpolate x4 and warpage 1 to remove spikes from dust. Then 3 pt height was selected in the primary plan view (Figure 16) and the result was recorded.

The measurements were repeated three times to check the reproducibility of the methods and to determine the standard deviations when assessing the sensitivity of the methods for detecting changes caused by the erosive challenge.

In Figure 17 the different surfaces of the scan are visible at 28 days where it was possible and the sample was large, a step to differentiate between the 14 and 28 days was attempted.

Figure 15 Grid view of a 14 day scan of a sample with A, B and C the three points of levelling

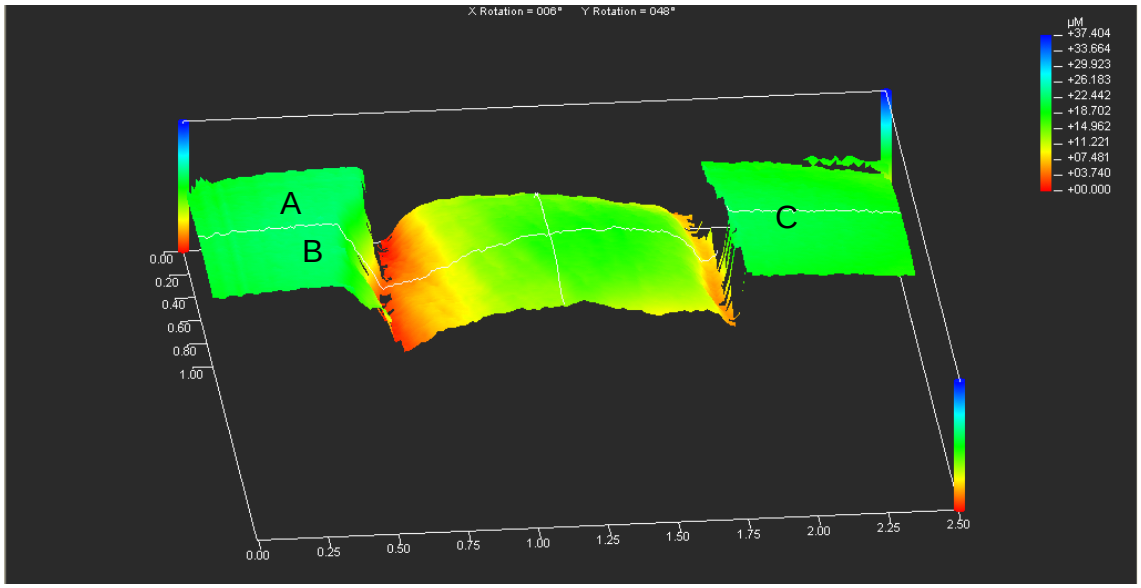


Figure 16 3pt height sample measurments with the result of the difference in height recorded at 13.603 µm

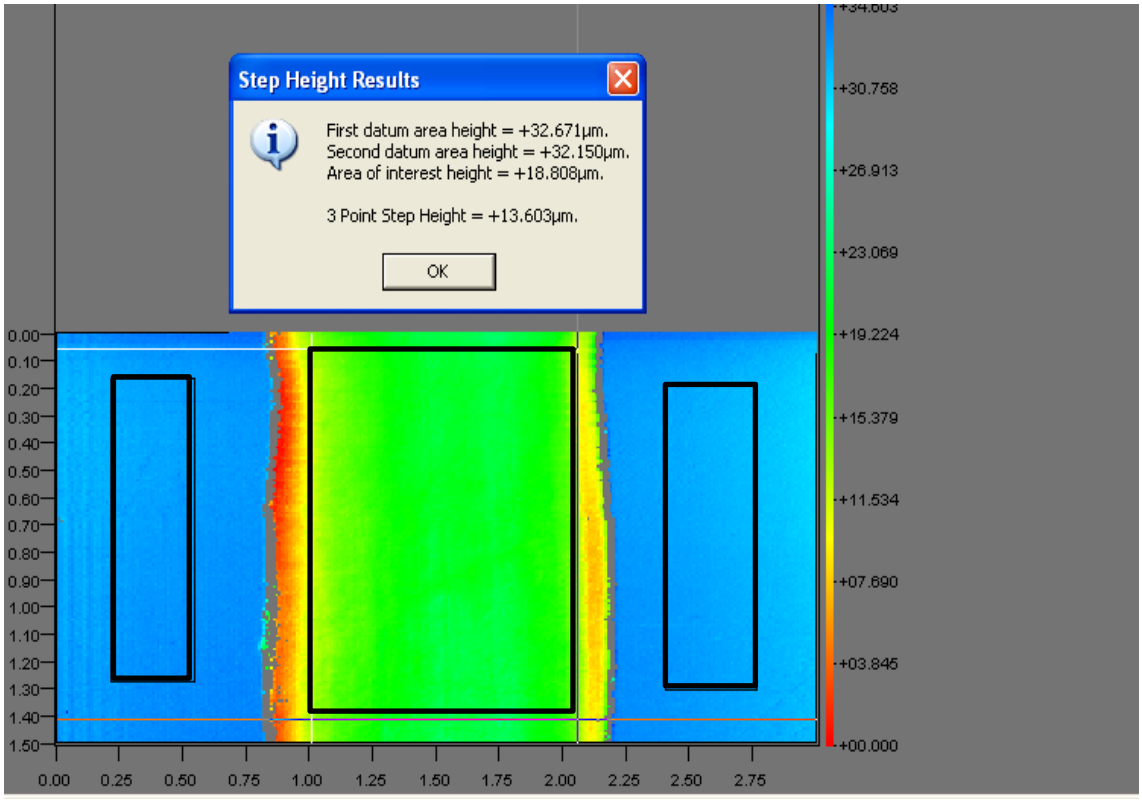
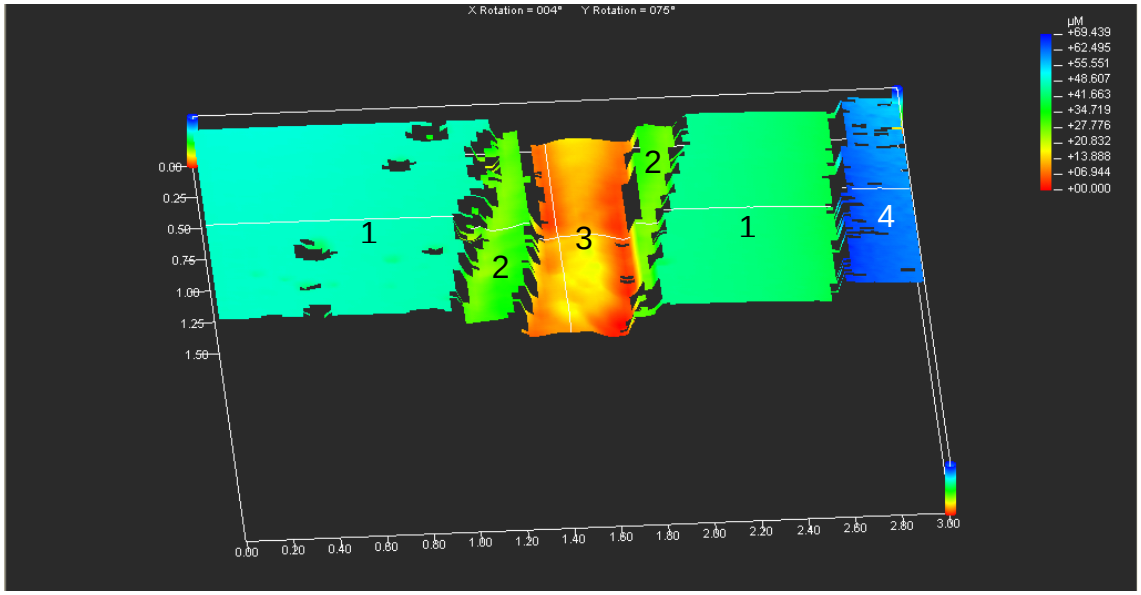


Figure 17 Grid view of at 28 days scan of a sample with 1 enamel intact surface , 2 step of erosive surface after 14 days, 3 bottom of erosive surface at 28 days and 4 the acrylic around the sample



3.3.7 Daily Fluoride release analysis

Three FGSRD from groups A and B were placed in centrifuge tubes with day time artificial saliva.

The FGSRD were placed in tubes with an equal amount of TISAB and the fluoride concentration measured using a Metrohm fluoride ion-specific electrode and Metrohm 781 Ion analyser after every 24h for 28 days.

Metrohm fluoride ion-specific electrode and Metrohm 781 analyser were used to determine the fluoride concentration of 3 slow release fluoride devices and 3 placebo devices after being diluted with Total Ionic Strength Adjustment Buffer (TISAB). The testing equipment was calibrated using fluoride standard solutions containing 0.01, 0.10, 1.00 and 10.00 mg/L F.

Figure 18 Metrohm 781 Ion analyser with the fluoride standards

3.4 Statistics

For the analysis, the data were uploaded in a SPSS Version 20. When the slabs were analysed the investigator did not know to which group they belonged to, making the analysis completely blinded.

The normality of the data was tested in order to proceed with the appropriate analysis.

For comparison of normal data t-tests were used with 0.05 as the significance level and a t-test, with a Bonferonni correction for comparisons within the groups.

4 Results

In this chapter the results of the study are presented. The presentation of the results is in the same order as described in materials and methods section in order to simplify reading.

4.1 Baseline Measurements

4.1.1 Profilometry measurements

Baseline measurements of the surface profile of the slabs were acquired using a surface profilometer (Scantron ProScan 2000) to ensure that the average depth range was (mean ISO Rz) less than 1.5 µm. If ISO Rz was s <1.5 the slab was deemed acceptable to be included in the study. Samples not within the parameters were reground and rescanned and if the second scan was within the measurements then the samples were given a code.

4.1.2 Microhardness measurements

Baseline measurements were recorded using Knoop microhardness. Microhardness was assessed using a computer-aided Duramin Indenter Machine (Struers A/S, DK 26-10, Denmark).

As can be seen in Table 6 the mean distance between the edges of the Knoop microhardness diamond was measured at 64.2 μm for the permanent enamel slabs and 64.55 μm for primary teeth with standard deviations of 2.21 and 2.14 respectively.

Samples not within the range were not recorded and discarded.

Table 6 Means of baseline microhardness measurements

Microhardness in permanent enamel slabs	
Mean	64.2 μm
Standard Deviation	2.21
Microhardness in primary enamel slabs	
Mean	64.55 μm
Standard Deviation	2.14

In Appendice 5 and 6 all the accepted measurements can be seen for the permanent and primary enamel slabs.

4.2 Tooth surface loss in permanent enamel

Data taken from groups A and B were analysed and both groups were normally distributed so an independent t test was used for the analysis of the groups. Table 7 presents the summary of the surface loss and Figure 19 is a boxplot that shows that both permanent groups were normally distributed.

Normality for the group A (placebo) and group B (fluoride) devices is presented in Table 8where the data are normally distributed.

In Appendices 7 to 10 all the measurements can be seen for the permanent enamel slabs.

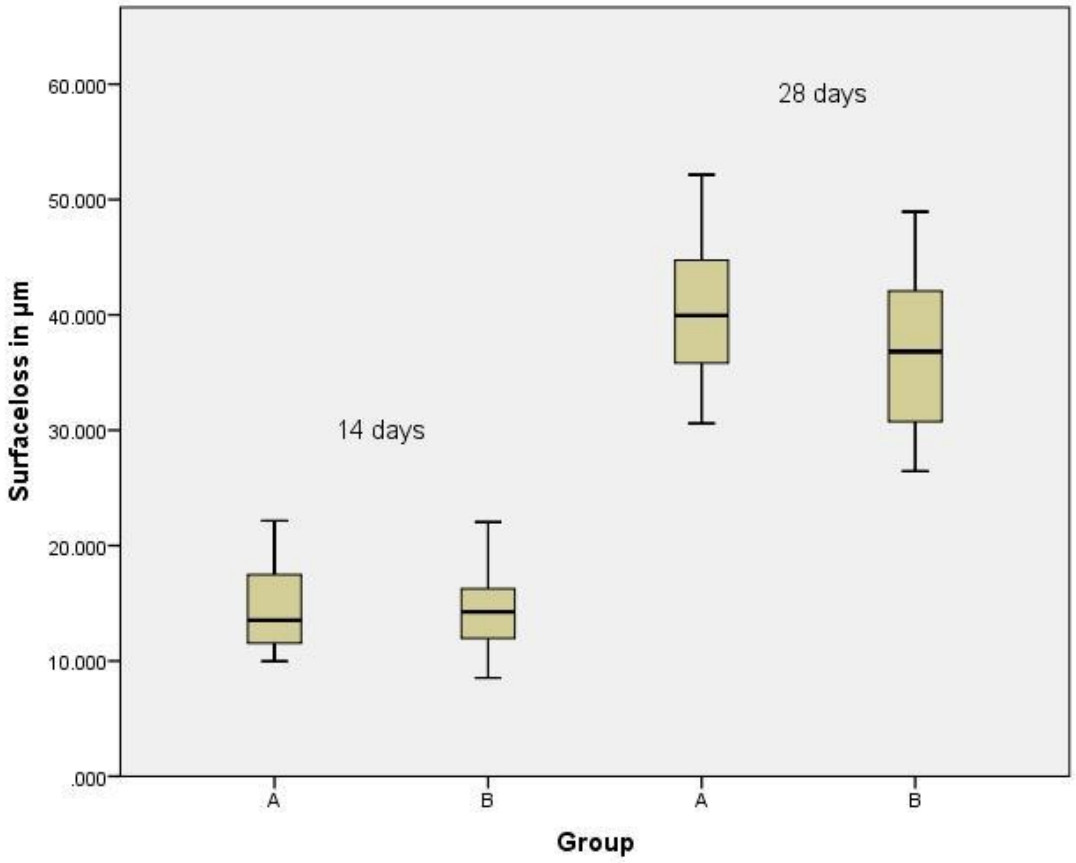
Table 7 Means of tooth surface loss (µm) for permanent enamel slabs after 14 and 28 days for Placebo and FGSRD groups.

SL Means± SE	*14days*	*28days*
Placebo	14.506 ± 0.7	40.108 ± 1.11
FGSRD	14.302 ± 0.6	37.005 ± 1.4
Difference	0.204	3.103

Table 8 Results of normality test for permanent enamel slabs

Test of Normality	Kolomogorov-Smirnov			Shapiro-Wilk		
Permanent enamel slabs	statistic	Df.	Sig.	statistic	Df.	Sig.
Placebo 14d	.183	24	.036	.926	24	.077
FGSRD 14d	.094	24	.200	.986	24	.978
Placebo 28d	.121	24	.200	.978	24	.855
FGSRD 28d	.122	24	.200	.950	24	.270

Figure 19 Box plots of surface loss for permanent enamel slabs placebo group (A) and Fluoride group (B) after 14 and 28 days



4.2.1 Descriptive statistics after 14 days

In Appendices 7 and 8 all the measurements can be seen for the permanent enamel slabs after 14 days. In the following Table 9 and 10 the results from the descriptive statistics are presented.

Table 9 Descriptive statistics of placebo devices for the permanent enamel slabs at 14 days

Group A Placebo devices for the permanent enamel slabs at 14 days	
Number of slabs	24
Median	13.53 µm
Minimum	9.98 µm
Maximum	22.15 µm
Range	12.18
Std. Deviation	3.41
95% Confidence interval for mean	13.06 µm Lower Bound 15.95 µm Upper Bound

Table 10 Descriptive statistics of FGSRD devices for the permanent enamel slabs at 14 days

Group B FGSRD for the permanent enamel slabs at 14 days	
Number of slabs	24
Median	14.26 µm
Minimum	8.53 µm
Maximum	22.04 µm
Range	13.51
Std. Deviation	3.2
95% Confidence interval for mean	12.94 µm Lower Bound 15.65 µm Upper Bound

4.2.2 Independent t-test at 14 days

Table 11 shows the surface loss comparison for the permanent enamel slabs at 14 days with significance p>0.05 (p=0.832)

Table 11 Comparing means of surface loss in permanent enamel slabs after 14 days

		T- TEST FOR EQUALITY OF MEANS			
		Sig.(2-tailed)	Std. Error Difference	95% Confidence Interval of the difference	
				Lower	Upper
SURFACE-LOSS A-B	Equal variances assumed	0.832	0..956	-1.72054	2.1289
	Equal variances not assumed	0.832	0.956	-1.72074	2.1291

4.2.3 Descriptive statistics after 28 days

In Appendices 9 and 10 all the measurements can be seen for the permanent enamel slabs after 28 days. The results from the descriptive statistics are shown in Tables 12 and 13.

Table 12 Descriptive statistics of placebo devices for the permanent enamel slabs at 28 days

Group A Placebo devices for the permanent enamel slabs at 28 days	
Number of slabs	24
Median	39.95 µm
Minimum	30.6 µm
Maximum	52.16 µm
Range	21.56
Std. Deviation	5.46
95% Confidence interval for mean	37.79 µm Lower Bound 42.41 µm Upper Bound

Table 13 Descriptive statistics of FGSRD devices for the permanent enamel slabs at 28 days

Group B FGSRD for the permanent enamel slabs at 28 days	
Number of slabs	24
Median	36.91 µm
Minimum	26.46 µm
Maximum	48.96 µm
Range	22.5
Std. Deviation	6.87
95% Confidence interval for mean	34.1 µm Lower Bound 39.9 µm Upper Bound

4.2.4 Independent t-test at 28 days

Table 14 shows the surface loss comparison for the permanent enamel slabs at 28 days with significance p>0.05 (p=0.091)

Table 14 Comparing means of surface loss in permanent enamel slabs after 28 days

		T- test for Equality of Means			
		Sig.(2-tailed)	Std. Error Difference	95% Confidence Interval of the difference	
				Lower	Upper
Surface loss A-B 28 day	Equal variances assumed	0.090	1.792	-0.5063	6.71076
	Equal variances not assumed	0.091	1.792	-0.5113	6.71572

4.3 Tooth surface loss in primary enamel

Data taken from groups C and D were analysed and both groups were normally distributed so an independent t test was used for the analysis of the groups. Table 15 presents the summary of the surface loss and Figure 20 is a boxplot that shows that both primary groups were normally distributed.

Normality for group C (placebo) and D (fluoride) devices is presented in Table 16 where the data were normally distributed.

In Appendices 11 to 14 all the measurements can be seen for the primary enamel slabs.

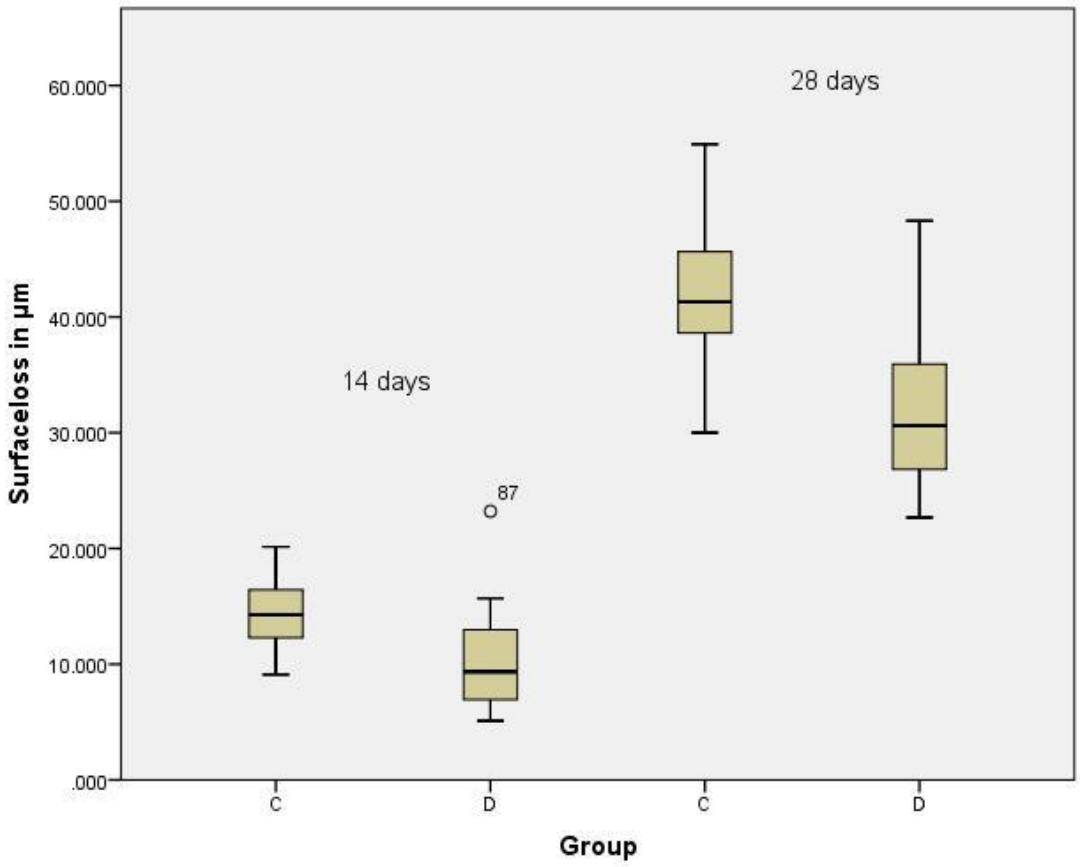
Table 15 Means of tooth surface loss (µm) for primary enamel slabs after 14 and 28 days for placebo and FGSRD groups

SL Means ± SE	*14days*	*28days*
Placebo	14.520 ± 2.2	42.236 ± 1.6
FGSRD	10.371 ± 4.362	32.116 ± 1.6
Difference	4.149	10.12

Table 16 Results of normality tests for primary enamel slabs

Test of Normality	Kolomogorov-Smirnov			Shapiro-Wilk		
Primary enamel slabs	statistic	Df.	Sig.	statistic	Df.	Sig.
Placebo 14d	.090	20	.200	.985	20	.981
FGSRD 14d	.160	20	.194	.879	20	.017
Placebo 28d	.231	20	.006	.919	20	.093
FGSRD 28d	.166	20	.148	.936	20	.201

Figure 20 Box plots of surface loss for primary enamel slabs placebo group (C) and Fluoride group (D) after 14 and 28 days



4.3.1 Descriptive statistics after 14 days

In Appendices 11 and 12 all the measurements can be seen for the primary enamel slabs after 14 days. The results from the descriptive statistics can be seen in Tables 17 and 18

Table 17 Descriptive statistics of placebo devices for the primary enamel slabs at 14 days

Group C Placebo devices for the primary enamel slabs at 14 days

Number of slabs	20
Median	14.26 µm
Minimum	9.1 µm
Maximum	20.14 µm
Range	11.04
Std. Deviation	2.92
95% Confidence interval for mean	13.15 µm Lower Bound 15.89 µm Upper Bound

Table 18 Descriptive statistics of FGSRD devices for the primary enamel slabs at 14 days

Group D FGSRD for the primary enamel slabs at 14 days

Number of slabs	20
Median	9.34 µm
Minimum	5.11 µm
Maximum	23.21 µm
Range	18.1
Std. Deviation	4.36
95% Confidence interval for mean	8.32 µm Lower Bound 12.41 µm Upper Bound

4.3.2 Independent t-test at 14 days

Table 21 shows the surface loss comparison for the primary enamel slabs at 14 days with significance p<0.05 (p=0.001).

Table 19 Comparing means of surface loss in primary enamel slabs after 14 days

		T- test for Equality of Means			
		Sig.(2-tailed)	Std. Error Difference	95% Confidence Interval of the difference	
				Lower	Upper
Surface loss C-D at 14day	Equal variances assumed	0.001	1.174	1.77136	6.5269
	Equal variances not assumed	0.001	1.74	1.76009	6.53817

4.3.3 Descriptive statistics after 28 days

In Appendices 13 and 14 all the measurements can be seen for the primary enamel slabs after 28 days. The results from the descriptive statistics are shown in Tables 20 and 21.

Table 20 Descriptive statistics of placebo devices for the primary enamel slabs at 28 days

Group C Placebo devices for the primary enamel slabs at 28 days

Number of slabs	20
Median	41.30 µm
Minimum	30.01 µm
Maximum	54.92 µm
Range	24.91
Std. Deviation	7.11
95% Confidence interval for mean	38.90 µm Lower Bound
	45.56 µm Upper Bound

Table 21 Descriptive statistics of FGSRD devices for the primary enamel slabs at 28 days

Group D FGSRD for the primary enamel slabs at 28 days

Number of slabs	20
Median	30.618 µm
Minimum	22.67µm
Maximum	48.31 µm
Range	25.64
Std. Deviation	7.164
95% Confidence interval for mean	28.766 µm Lower Bound
	35.472 µm Upper Bound

4.3.4 Independent t-test 28 days

Table 22 shows the surface loss comparison for the primary enamel slabs at 28 days with significance $p<0.05$ ($p=0.001$).

Table 22 Comparing means of surface loss in primary enamel slabs after 28 days

		T- test for Equality of Means			
		Sig.(2-tailed)	Std. Error Difference	95% Confidence Interval of the difference	
				Lower	Upper
Surface loss C-D 28 day	Equal variances assumed	0.000	2.257	5.5473	14.6857
	Equal variances not assumed	0.001	2.257	5.5473	14.6858

4.4 Intra-examiner correlation

From all the enamel slabs measurements (n=88) 20 enamel slabs were chosen 5 from each group and they were reanalysed. Appendix 15 has the means of these measurements for both 14 and 28 days. In Table 23 the results of intra-class correlation coefficient are presented with the correlation above 98% for both 14 and 28 days.

Table 23 Results of intra-class correlation coefficient

		Intra-class correlation coefficient		
		Inta-class correlation	95% Confidence Interval of the difference	
			Lower	Upper
14 days	Single Measurements	.986	.965	.994
	Average Measurements	993	.993	.982
28 days	Single Measurements	.993	.950	.998
	Average Measurements	.997	.974	.999

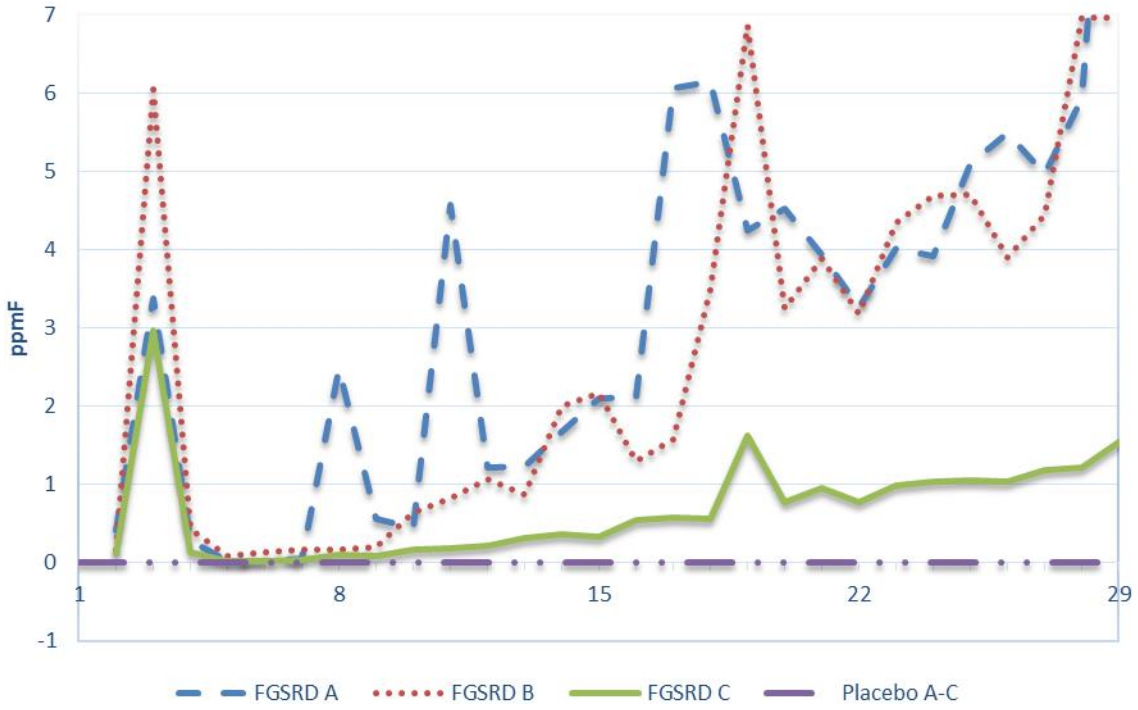
4.5 Daily fluoride release of the FGSRD devices

Metrohm fluoride ion-specific electrode and Metrohm 781 analyser were used to determine the fluoride concentration of 3 slow release fluoride devices and 3 placebo devices after being diluted with TISAB. Metrohm fluoride ion-specific electrode measurements are presented in Table 24 but only for the FGSRD the measurements for the placebo devices could not be recorded since the electrode was not sensitive to measure the amount of F smaller than 0.01ppmF, which was the lowest concentration standard. In Figure 21 the amount of F in ppmF is displayed that was released from the 3 FGSRD's (A-C) and the placebo devices (A-C). The detailed readings of F are shown in Appendix 16.

Table 24 Results after the analysis of fluoride devices

Devices	*FGSRD A*	*FGSRD B*	*FGSRD C*
Mean (ppmF)	3.32	2.08	0.554
Std Deviation	2.683	2.313	0.661

Figure 21 Fluoride readings for 28 days for the FGSRD



5 Discussion

5.1 Introduction

The aim of this chapter is to explain the effect of FGSRD's in preventing erosion in human teeth in vitro. In order to formulate the study design the following were needed:

- An erosive abrasion technique that simulated the oral conditions
- Measuring tools capable of assessing tooth surface loss
- Sample size calculation for permanent and primary teeth

The study design that was used was:

- Erosive abrasion model with 5 dipping's/2min in citric acid and 15 strokes/2min brushing
- Surface profilometry measurements

5.2 Justification of study aims

Erosive tooth wear is a condition that has become more prominent in recent years with Birgit Angmar-Mansson having described it as a ″challenge for the 21st century″ (Lussi and Ganss, 2014). Dental erosion is multifactorial and the management of the condition is usually when it has progressed to the need for restorations. Prevention is usually personalised instructions when the tooth surface loss has progressed and is visible. A method that helps preventing tooth loss is needed. FGSRD's have been tested and found beneficial in decreasing dentinal hypersensitivity (Malik-Kotru, 2009), increasing plaque fluoride levels (Abudiak, 2007) and prevention of orthodontic white spot lesion demineralisations (Tatsi, 2014). They can be easily attached to the teeth and the duration of release of F and the concentration in saliva was studied by Bashir, 1988 reporting that F was released for a prolonged period of 18 months (Toumba, 2000).

Fluoride is the foundation of prevention and remineralisation, and many methods of F application and supply to the tooth surface have been developed. The preventive effect of F is predominantly by its topical rather than its systemic effect and it is patient cooperation dependent.

The effect of FGSRD's on the prevention of dental erosion has never been investigated so this is the reason that in this research project FGSRD's were tested under an erosive challenge with human primary and permanent dental enamel in vitro.

5.3 Appraisal of the methodology

An in vitro erosion abrasion model was used to investigate the effect of the FGSRD on tooth surface loss of human enamel under erosive conditions. In testing the devices this study attempted to replicate an erosive challenge that could simulate the conditions in the mouth for the duration of 28 days.

In vitro models are useful because they can be executed over a short period of time, are not too costly, they do not rely on participants' compliance as for in situ studies. They are also not harmful as no subjects are involved and therefore for the participants so ethical approval is not required and they do not need a lot of staff to run them.

The limitations are that they cannot replicate completely all of the variables in the oral cavity that lead to erosion. The amount of surface loss can be measured by many techniques but the most suitable has been recorded to be profilometry and microradiography (Schlüter et al., 2011).

In previous in vitro studies the hard tissue that was used (bovine or human), frequency of acid attacks, and duration of dipping and the medium of erosive challenge varied.

The present study decided to use human teeth in preference to bovine teeth since the study was testing the FGSRD's and placebo devices in permanent and primary teeth. In this study a modification of an erosive challenge that had been used successfully for the investigation of enamel demineralisation in many research studies (Abdullah, 2009).

A five daily dipping regime in citric acid was used for two minutes for each application and brushing two times daily with fluoride-free tooth paste. The present study tried to simulate the scenario that a participant would have five meals with an acid drink and brush their teeth morning and night.

The difference from other models was that this study did not wait one hour after the last dipping in citric acid as the enamel slabs were rinsed and brushed immediately and that may have an effect making the surface loss more severe. Lussi and Carvalho (2014) stated that the effect of acid took longer than one hour to be neutralised. By doing that we had the opportunity to test the devices under more severe conditions was possible.

5.4 Evaluation of the results

This is an exploratory study using an erosive abrasion model to test the preventive properties of FGSRD against the erosive action of citric acid on the surface of permanent and primary human teeth. The prevalence of erosion is increasing in recent years in all age groups. There is no reported data demonstrating the effect of any fluoride slow release devices for the prevention of erosion. From the studies we found the use of F for prevention of erosion has been tested and the results were either of low significance or the preventive effect was for a limited time of up to 4h and again with low level significance of the results (Wegehaupt et al., 2012).

Ganss et al. (2004) explained that F has two actions in erosion protection, it first reacts with the acid and creates a protective layer of CaF_4, the second is again the formation of a protective layer against erosive substances due to the action of metal based F (Schlueter et al., 2009, Büyükyilmaz et al., 1997, Büyükyilmaz et al., 1994).

None of the studies found in the literature used materials that released very small amounts of F similar to the FGSRD and even the other slow-release devices have not been tested against an erosive challenge. Currently FGSRD's distribution awaits permit from the American food and drug administration. From the studies of Murakami et al. (2009) that used both primary and permanent teeth while testing varnishes and gels. Results showed that prevention of primary enamel erosion by fluoride was not significant while permanent enamel showed a significant effect. Interestingly, in the present study the opposite results were

observed: for enamel of primary teeth, significant less erosion was observed in the F device group for the duration of the study (p<0.001However, for permanent enamel, no significant differences were observed (p>0.05).

5.4.1 Tooth surface loss in permanent enamel

From the analysis of the permanent enamel slabs n=48 (FGSRD: n=24 and non-FGSRD: n=24) there was no loss of slabs with a 100% completion rate for the duration of the 28 days.

The data were normally distributed for the duration of the study so an independent t-test to check the means was possible. After 14 days there was no significance between the means of the two groups p=0.432. The significance at 28 days showed a trend of reducing and coming close to $p<0.05$ but still was not significant p=0.091.

Surface loss with FGSRD after 28 days was around 36.91µm (26.46 - 48.96 µm) and was not significant in comparison with the placebo devices effect on surface loss which was 39.95 µm (30.95 - 52.16 µm).

From the results of this study the effect of the FGSRDs for the protection of permanent teeth cannot be determined. Other studies testing the effect of fluoride products with higher concentrations of F showed promising results in the erosion reduction between test and controlled groups (see chapter 2.2.1). A trend was observed that indicated that if the study was to either continue for several more days or the sample size was larger there would probably have been a significant difference.

The difference between F and non-F devices after 14 days was almost -0.2µm and after 28 days was -3.1µm which was approaching clinical significance but did not reach it. Further research is necessary to clarify if there is a significant effect of the slow-release devices on the prevention of erosion in human permanent teeth.

5.4.2 Tooth surface loss in primary enamel

After the analysis of the primary enamel slabs n=40 (FGSRD: n=20 and non-FGSRD: n=20) there was no loss of slabs with a 100% completion rate for the duration of the 28 days of the study cycling regime.

The data were normally distributed for the duration of the study so an independent t-test to check the means was possible. After 14 days there was significance between the means of the two groups $p=0.001$ $p<0.05$. The significance at 28 days was also significant $p=0.000$.

Surface loss with FGSRD after 28 days was 30.62 µm (22.67 – 84.31 µm) and when compared with the placebo devices where the surface loss was 41.30 µm (30.01 – 41.30 µm) a 31% difference was observed. That difference was even visible when the data were analysed after 14 days of cycling with a 40% difference in the surface loss on the slabs with FGSRD's.

Only a few studies have tested the preventive effects of F products on erosive challenges in the primary dentition. Murakami et al. (2009) showed that prevention of primary enamel erosion by fluoride gels and varnishes was not significant. In the current study the results looked very promising since the difference in the mean erosion surface depth was 10.12µm less in the F group than in the non-F group.

According to Shellis (1984) primary teeth have more porous enamel prisms and this may also be a reason that the low concentrations of fluoride in the FGSRDs were effective. Also Sønju Clasen and Ruyter (1997) found that primary dental enamel had more carbonate ions replacing hydroxyl and phosphate groups. The formation of Type A and B carbonate hydroxyapatite was not as tightly bound, as that of permanent enamel, making the enamel in primary teeth more soluble (Sønju Clasen and Ruyter, 1997). Therefore, the solubility differences between primary and permanent enamel may explain the observation of significant results in the primary but not the permanent enamel groups

5.4.3 Daily fluoride release of the FGSRD devices

Three glass devices from the two groups were placed in centrifuge tubes with day time artificial saliva that was used in the experiment.

The glass devices were placed in tubes with an equal amount of TISAB and the fluoride concentration measured using a Metrohm fluoride ion-specific electrode and Metrohm 781 Ion analyser after every 24h for 28 days.

Metrohm fluoride ion-specific electrode and Metrohm 781 analyser were used to determine the fluoride concentration of three slow release fluoride devices and three placebo devices after being diluted with total ionic strength adjustment buffer (TISAB). The testing equipment was calibrated using fluoride standard solutions containing 0.01, 0.10, 1.00 and 10.00 mg/L F.

It was not possible to record if there was any F in the placebo devices since measurements below 0.01ppmF were recorded as “error”. The mean release from the FGSRD’s was from 0.554 - 3.32 ppmF for each day. During the first days there was a surge of F release that was explained by Attar and Turgut (2003) and Karantakis et al. (2000) and is due to the action of the material that releases the high concertation within the few first days and then it balances. The same effect was observed in previous studies testing FGSRD’s where the concentration increased up to the third day and then was constant (Malik-Kotru, 2009).

In the present study the fluoride release in the artificial saliva at the beginning gradually increased until the second day, then decreased and then was constant for a few days. However, FGSRD A and B followed an inconsistent daily F release that was probably due to the fracture of the devices and therefore an increase in surface area which resulted in an increased release of F. FGSRD C followed a more uniform rate of F release with a mean value of 0.554 ppmF.

5.5 Study limitations and challenges encountered

During the data analysis and the final consultation with the statistician it became apparent that surface loss comparisons between primary and permanent teeth could not be calculated. Primary and permanent teeth not only have different morphologies and anatomy but also differences in their mineral and histological compositions.

Primary teeth are smaller and have a thinner enamel layer than permanent teeth (Grine, 2005) which influences their weakness towards erosive attack. The ion composition of primary teeth differs and is less mineralised than permanent teeth (Wilson and Beynon, 1989). There are a number of studies comparing the susceptibility of primary teeth to permanent teeth. Amaechi et al. (1999) found a significantly greater mineral loss when dipping teeth in orange juice. Attin et al. (2007) on the other hand reported that there was no significant difference between primary and permanent human teeth.

In general epidemiological studies have reported that primary teeth are softer and easier to erode than permanent teeth (Kreulen et al., 2010).
Carvalho et al. (2014) proposed that different factors influenced erosion in humans and it cannot be replicated completely in the laboratory.

In the daily F analysis the devices gave inconsistent results which may have been due to the fracture of the devices. The devices fractured when they were moved from one centrifuge tube to another with metal tweezers. Fracturing the devices altered the surface area of the devices thus influencing the results for daily fluoride release.

5.6 Recommendations for future research

Data from this study can be used for power calculations to determine the sample size for future studies especially for an in situ study.

The results from the analysis of the permanent enamel slabs were not significant so it was decided to recalculate the sample size using the results of this study. It was determined that doubling the number of slabs used was needed to see any significance. A repetition of the experiment using more permanent teeth may be beneficial.

Due to inconsistent results of daily F release from the FGSRD a specific study to check the daily and long term F release is advised. An investigation of how the surface area affects the release of F in distilled water, artificial saliva or even in pooled saliva is required.

6 Conclusions

From the results demonstrated in this study it can be concluded that the null hypotheses that were made originally are either accepted or rejected as follows:

Hypothesis 1 (Accepted): There was no difference between test and placebo glass slow-release devices on the prevention of an erosive challenge on the dental enamel of permanent teeth.

Hypothesis 2 (Rejected): There was no difference between test and placebo glass slow-release devices on the prevention of an erosive challenge on the dental enamel of primary teeth.

There was a difference in the results for the surface loss of enamel of primary teeth. After 14 days, 40% less erosion was observed in the F group compared to the placebo group which decreased to 31% at the end of the study period, i.e., 28 days.

Hypothesis 3 (Void): There is no difference in the prevention properties of the FGSRD between permanent and primary enamel.

Surface loss comparisons between primary and permanent teeth could not be calculated (see study limitations).

FGSRDs have great potential for protection of primary human enamel against erosion. To confirm this more research studies are needed and the devices should also be compared to other preventive products.

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8 List of Appendices

Version 3 28 January 2014

Application form and proposed Standard Operating Procedure for use of tissue stored in the School of Dentistry Skeletal Tissues Bank

1. Application for use of Tissue from the School of Dentistry Skeletal Tissues Bank

Title of project:

The effect of fluoride glass slow-release devices on the protection of primary and permanent dental enamel to erosive challenge

Application number: 300114/GK/123

Lead applicant name: Gioula Kotantoula

Co-applicant name(s) including supervisor(s):
KJ Toumba
MS Duggal

What samples do you require and how many?
Permanent teeth: 15 Molars or 25 Premolars with sound surfaces.
Primary teeth: 30 Molars with sound surfaces.

Brief outline of aims and objectives in lay language:

Aim: The investigation of the use of fluoride glass slow-release devices for the protection of dental erosion of human dental enamel in-vitro

Objectives: To use the fluoride glass slow-release devices for prevention of against erosion in primary and permanent teeth.

Is this work part of an UG project?

NO

Is this work part of a PG project?

YES

If YES give the degree Title:

Professional Doctorate in Paediatric Dentistry

Is it intended that any tissue, or parts of tissue (including cells grown on from primary tissue samples) will be sent outside of Leeds University/Teaching Hospitals NHS Trust?

NO

2. Standard Operating procedure for use, storage and disposal of tissue in this project:

Where will the work be carried out?
Lab at Level 6 Leeds Dental School

How will the tissue be stored?
The slabs will be kept moist in distilled water and left at room temperature to prevent dehydration.

How will the tissue be disposed of?
After obtaining required data enamel slabs will be appropriately disposed of through the LDI clinical waste system (after giving a notice).

How is the proposed work to be funded?
Professional Doctorate Research Fund

How was the work scientifically reviewed?
By the Child Dental Health (CDH) and DREC

How will the results of the work be disseminated?
Presentations in conferences.
Thesis for Professional Doctorate in Paediatric Dentistry.
Publication in Dental journals.

Please attach a brief description of the proposed work, highlighting the methods to be used.

I confirm that the proposed work meets with the requirements of the Leeds Skeletal Tissue Bank Policy and that the tissue released under the remit of this SOP will be used for no other purpose.

Lead Researcher (print name): Gioula Kotantoula

Signature: Kotantoula Gioula

Date: 10/04/2014

Authorisation of approval:

Approval given by (print name):

Signature: .....

Date: .....

Appendix 2. Approval email from Tissue Bank

To: Gioula Kotantoula;

Cc: David Wood; Jack Toumba; Monty Duggal;

Dear Gioula

I am pleased to inform you that the above Tissue Bank application has been accepted by the Dental Research Ethics Committee.

Documents reviewed by the Committee

Document name	Version number and date
Protocol	Version 1 30/01/2014

Please note that teeth will be supplied from the dried collection. If numbers need to be topped up from fresh, these will be issued on an available basis with other users of the Bank.

With best wishes for the success of your project.

For and on behalf of
Professor David Wood
Deputy DREC Chair

Appendix 3. Tissue Sample form for permanent teeth

UNIVERSITY OF LEEDS

The Leeds Teaching Hospitals NHS
NHS Trust

Tissue Sample Form for School of Dentistry Research Tissue Bank

Applicant: Gioula Kotantoula

DREC No: 300114/GK/123

Research Tissue: TEETH

Bank ID No: 15 x ~~Pre~~ MOLARS

Age of patient (time of collection):

Sex:

Ethnic Origin:

Signed (Recipient): Kotantoula Gioula

Signed (Supplier): J. Hobson

Date sample taken: 18/7/14

211113|2|b.
211112|2|d
210213|1|a
051113|1|a
130214|2|a
020913|1|c
230614|1|c
020513|1|a
040614|1|a
290513|1|d
130813|1|a
070313|1|a
040313|1|b
290513|1|c
120313|1|d.

Please ensure Bank ID number is recorded on all samples created from tissue received from the Tissue Bank.

This is a requirement of the Human Tissue Act. By receiving tissue from the bank you are agreeing to abide by the HTA regulations.

Useful links:
http://www.hta.gov.uk/ Human Tissue Authority website
http://www.opsi.gov.uk/acts/acts2004/ukpga_20040030_en_1 Human Tissue Act 2004

Appendix 4. Tissue Sample form for primary teeth

14 July 2014.

Gioula Kotantoula. Paediatric Dentistry.

Primary teeth.

1) 151013/2/a ✓
2) 0402 14/1/c ✓
3) 140313/1/b ✓
4) 040214/1/b ✓
5) 250214/2/a ✓
6) 030313/2/a ✓
7) 110314/1/a ✓
8) 100614/1/b ✓
9) 230713/1/c ✓
10) 180713/1/a ✓
11) 230713/1/b ✓
12) 271113/1/c ✓
13) 230713/1/a ✓
14) 151013/1/b ✓
15) 100614/1/a ✓
16) 191113/1/a ✓
17) 151013/1/d ✓
18) 271113/1/e ✓
19) 271113/1/b ✓
20) 191113/1/b ✓
21) 140313/1/c ✓
22) 151013/1/c ✓
23) 210611/1/d ✓
24) 060311/2/b ✓
25) 060311/2/a ✓
26) 210611/1/c ✓
27) 100712/2/a ✓
28) 111113/a ✓
29) ~~261012/1/b~~ 261012/1/b ✓
30) 211112/1/a ✓
31) 190612/2/b ✓
32) 200213/2/b ✓
33) 200213/2/a ✓
34) 211011/1/c ✓
35) 211011/1/d ✓
36) 130612/3/a ✓

UNIVERSITY OF LEEDS

The Leeds Teaching Hospitals NHS Trust

Tissue Sample Form for School of Dentistry Research Tissue Bank

Applicant: Gioula Kotantoula

DREC No: 300114/GK/123

Research Tissue: .....

Bank ID No: See attached sheet X 36 Primary

Age of patient (time of collection):.....

Sex: .....

Ethnic Origin:.....

Signed (Recipient): Kotantoula Gioula

Signed (Supplier): T. Kodan

Date sample taken: 18/7/14

Please ensure Bank ID number is recorded on all samples created from tissue received from the Tissue Bank.

This is a requirement of the Human Tissue Act. By receiving tissue from the bank you are agreeing to abide by the HTA regulations.

Useful links:
http://www.hta.gov.uk/ Human Tissue Authority website
http://www.opsi.gov.uk/acts/acts2004/ukpga_20040030_en_1 Human Tissue Act 2004

Appendix 5. Microhardness readings (µm) from permanent enamel slabs

Slabs ID	M1	M2	M3	Mean Perm
1	62.6	68.4	66.5	66.5
2	67.3	68.4	67.8	67.8
3	66.7	67.8	68.7	67.8
4	67.3	67.6	68.7	67.6
5	64.8	67.6	65.6	65.6
6	64.8	60.4	66.7	64.8
7	67.8	65.9	69.5	67.8
8	67.6	63.1	63.4	63.4
9	62.3	65.3	67.3	65.3
11	61.7	62.9	60.4	61.7
12	61.5	63.4	60.9	61.5
13	61.7	60.6	61.2	61.2
14	64.2	62.3	64	64
15	65.6	69.8	63.7	65.6
16	69.2	64.2	61.2	64.2
17	65.3	65.1	64.8	65.1
18	63.7	63.1	64.2	63.7
19	66.7	65.9	67.8	66.7
20	69.2	68	65	68
21	61.5	61.7	62.6	61.7
22	62	64.2	67.3	64.2
24	68.7	63.1	60.6	63.1
25	64	67	62.9	64
26	65.3	63.1	64	64
27	64.8	65.8	62.9	64.8
28	64.5	64.5	66.5	64.5
29	62.3	62	63.4	62.3
30	64	65.6	64.5	64.5
31	61.5	60.1	63.7	61.5
32	65.9	68.9	66.2	66.2
33	64	65.6	67.3	65.6
38	68.1	66.5	60.4	66.5
39	60.9	61.2	65.1	61.2
40	68.9	69.8	69.2	69.2
41	64.8	69.2	65.3	65.3
42	62.6	60.4	60.6	60.6
43	65.3	68.1	65.9	65.9
A1	65.7	61.1	60.2	61.1
A2	63.9	69.74	68.76	68.76
A3	65.7	63.9	62	63.9
A4	67.8	67.6	68.7	67.8
B1	63.7	64	61.7	63.7
B2	62.3	68.1	57.9	62.3
C1	61.5	64.2	65.6	64.2
C2	68.4	66.7	65.9	66.7
E1	60.4	61.2	65.1	61.2
E3	64.2	66.5	62.6	64.2
E4	67.6	64	63	64

Appendix 6. Microhardness readings (µm) from primary enamel slabs

Slab ID	M1p	M2p	M3p	Mean Primary
1	67.3	69.5	69.8	69.5
2	59.8	60.1	62.9	60.1
3	64.5	66.7	61.5	64.5
4	65.1	66.2	68.9	66.2
5	64	60	66.5	64
6	64	62.3	63.4	63.4
7	67.6	69.8	67.6	67.6
8	64.8	65.9	61.7	64.8
9	69.2	64.8	64.5	64.8
11	62.3	60.1	65.3	62.3
13	64	60.9	62.3	62.3
14	67.6	62	61.7	62
15	63.1	61.2	64	63.1
17	64.5	67.6	66.2	66.2
18	64.8	65.1	68.9	65.1
19	67.6	64.2	62.9	64.2
21	64.5	67	64.2	64.5
22	64.8	63.7	64.8	64.8
23	65.3	64.8	65.1	65.1
24	63.4	68.2	61	63.4
25	60.4	64	67.2	64
28	60.3	60.6	65	60.6
29	61.4	67.9	69	67.9
30	64.4	66.8	67.2	66.8
31	61.7	64	66.5	64
33	65.1	60.6	62.8	62.8
34	62	62.1	61.3	62
35	66.5	63.7	60	63.7
36	66.2	64.7	66	66
37	62	60.8	61.8	61.8
38	68	62.1	66.1	66.1
39	64.8	60.3	65.2	64.8
40	63.9	62	67	63.9
41	62.8	64.7	69.4	64.7
42	61.3	65.8	62.9	62.9
45	68.7	67.2	66.1	67.2
46	67.9	66.5	67.1	67.1
47	68.3	67.1	67.6	67.6
49	69.9	68.5	67.3	68.5
50	64.7	63.3	64.6	64.6

Appendix 7. Readings for group A (non-FGSRD) after 14 days (µm)

AP slabs Days14	Reading 1	Reading 2	Reading 3	Mean	Stand Deviation
A3a	14.435	13.710	13.872	14.006	0.381
A4a	11.108	11.367	11.064	11.180	0.164
B2a	16.931	18.052	17.516	17.500	0.561
C2a	18.820	18.899	19.578	19.099	0.417
E2a	17.438	17.080	17.841	17.453	0.381
E3a	12.550	12.259	12.556	12.455	0.170
E4a	10.554	11.595	11.413	11.187	0.556
1a	21.538	22.234	22.692	22.155	0.581
2a	19.687	19.508	19.677	19.624	0.101
5a	14.279	13.696	13.710	13.895	0.333
7a	13.932	13.981	13.310	13.741	0.374
9a	12.920	13.070	12.984	12.991	0.075
11a	11.388	11.225	11.356	11.323	0.086
12a	10.648	10.577	10.958	10.728	0.203
21a	10.976	10.068	10.416	10.487	0.458
22a	11.831	11.696	11.771	11.766	0.068
25a	13.249	13.547	13.178	13.325	0.196
27a	9.997	10.800	9.136	9.978	0.832
32a	12.161	13.094	13.783	13.013	0.814
34a	12.635	12.850	12.332	12.606	0.260
38a	18.634	19.004	18.530	18.723	0.249
39a	16.033	15.910	16.017	15.987	0.067
40a	16.719	16.649	16.213	16.527	0.274
42a	18.450	18.500	18.234	18.395	0.141

Appendix 8. Readings for group B (FGSRD) after 14 days (µm)

BP slabs Days 14	Reading 1	Reading 2	Reading 3	Mean	Stand Deviation
A1a	11.476	12.081	11.561	11.706	0.328
A2a	13.826	14.045	14.015	13.962	0.119
B1a	14.786	14.613	15.087	14.829	0.240
C1a	10.898	10.841	10.995	10.911	0.078
E1a	13.851	14.745	14.021	14.206	0.475
3a	21.697	22.260	22.161	22.039	0.301
4a	19.125	19.222	19.312	19.220	0.094
6a	16.237	15.949	15.958	16.048	0.164
8a	18.299	17.902	18.316	18.172	0.234
13a	12.442	11.500	12.396	12.113	0.531
14a	8.279	9.441	7.859	8.526	0.819
16a	9.664	9.545	9.469	9.559	0.098
17a	15.134	13.769	14.035	14.313	0.724
18a	10.491	9.574	10.389	10.151	0.503
19a	19.295	12.136	17.241	16.224	3.686
20a	15.699	16.813	16.561	16.358	0.584
24a	18.092	17.258	18.243	17.864	0.531
26a	13.358	13.357	12.965	13.227	0.227
28a	12.923	11.318	11.232	11.824	0.952
29a	12.561	13.817	11.790	12.723	1.023
30a	14.738	15.667	14.689	15.031	0.551
31a	11.975	12.448	13.978	12.800	1.047
33a	16.069	16.382	16.476	16.309	0.213
41a	15.188	14.759	15.426	15.124	0.338

Appendix 9. Readings for group A (non-FGSRD) after 28 days (µm)

AP slabs Days 28	Reading 1	Reading 2	Reading 3	Mean	Stand Dev
A3a	41.982	41.723	40.249	41.318	0.935
A4a	32.616	30.377	32.521	31.838	1.266
B2a	44.150	44.513	45.266	44.643	0.569
C2a	45.532	46.480	46.228	46.080	0.491
E2a	48.669	46.272	46.281	47.074	1.381
E3a	38.769	39.665	38.012	38.815	0.827
E4a	35.310	36.185	36.165	35.887	0.500
1a	52.373	53.498	50.621	52.164	1.450
2a	40.423	40.607	40.754	40.595	0.166
5a	33.208	32.807	32.952	32.989	0.203
7a	40.965	40.842	41.46	41.089	0.327
9a	40.780	40.881	40.955	40.872	0.088
11a	27.827	33.74	30.236	30.601	2.973
12a	33.904	34.423	33.507	33.945	0.459
21a	44.776	38.085	38.413	40.425	3.772
22a	36.398	34.733	33.523	34.885	1.443
25a	40.49	39.116	38.832	39.479	0.887
27a	39.188	38.203	39.097	38.829	0.544
32a	35.542	36.059	35.719	35.773	0.263
34a	37.493	38.308	37.751	37.851	0.417
38a	45.751	45.303	46.647	45.900	0.684
39a	38.227	39.615	39.352	39.065	0.737
40a	47.811	47.88	47.151	47.614	0.402
42a	45.193	44.89	44.478	44.854	0.359

Appendix 10. Readings for group B (FGSRD) after 28 days (µm)

BP slabs Days 28	Reading 1	Reading 2	Reading 3	Mean	Stand Dev
A1a	30.797	32.429	31.271	31.499	0.840
A2a	27.417	29.13	29.616	28.721	1.155
B1a	39.436	35.737	36.376	37.183	1.977
C1a	28.187	26.997	29.278	28.154	1.141
E1a	39.748	40.445	39.439	39.877	0.515
3a	48.105	48.383	48.366	48.285	0.156
4a	48.683	49.691	48.491	48.955	0.645
6a	38.222	37.324	39.268	38.271	0.973
8a	48.871	48.271	48.286	48.476	0.342
13a	29.202	30.699	30.965	30.289	0.950
14a	29.658	27.407	29.339	28.801	1.218
16a	26.355	26.544	26.468	26.456	0.095
17a	43.118	43.123	42.837	43.026	0.164
18a	29.786	30.834	29.586	30.069	0.670
19a	35.244	34.666	36.542	35.484	0.961
20a	44.926	45.378	44.871	45.058	0.278
24a	43.122	44.318	43.553	43.664	0.606
26a	38.869	39.271	39.818	39.319	0.476
28a	39.864	38.731	39.310	39.302	0.567
29a	30.303	30.67	32.644	31.206	1.259
30a	36.518	34.519	34.429	35.155	1.181
31a	32.896	32.869	34.122	33.296	0.716
33a	41.268	41.239	40.833	41.113	0.243
41a	36.003	36.671	36.742	36.472	0.408

Appendix 11. Readings for Group C (non-FGSRD) after 14 days (μm)

CD slabs Days 14	Reading 1	Reading 2	Reading 3	Mean	Stand Deviation
1p	15.732	14.945	15.981	15.553	0.541
2p	14.516	13.723	13.116	13.785	0.702
5p	12.215	12.874	12.651	12.580	0.335
6p	17.733	16.556	14.636	16.308	1.563
7p	11.255	11.423	11.819	11.499	0.290
11p	12.554	13.202	12.780	12.845	0.329
12p	17.831	18.468	18.069	18.123	0.322
14p	13.543	13.810	13.192	13.515	0.310
15p	8.597	9.397	9.309	9.101	0.439
21p	16.704	16.432	16.539	16.558	0.137
22p	14.935	14.950	15.166	15.017	0.129
23p	11.132	12.297	12.511	11.980	0.742
24p	12.187	11.466	12.348	12.000	0.470
25p	11.336	10.597	9.925	10.619	0.706
31p	18.948	21.424	20.045	20.139	1.241
37p	13.898	14.500	13.159	13.852	0.672
38p	15.435	15.251	14.871	15.186	0.288
39p	15.035	14.135	14.883	14.684	0.482
40p	19.937	18.607	19.935	19.493	0.767
41p	17.818	17.020	17.860	17.566	0.473

Appendix 12. Readings for Group D (FGSRD) after 14 days (μm)

DD slabs Days 14	Reading 1	Reading 2	Reading 3	Mean	Stand Deviation
3p	9.691	10.626	7.606	9.308	1.546
4p	11.057	12.261	11.400	11.573	0.620
8p	21.597	23.007	25.024	23.209	1.722
9p	10.705	10.271	10.616	10.531	0.229
13p	6.943	7.530	9.753	8.075	1.482
17p	6.925	6.623	6.571	6.706	0.191
18p	16.385	12.496	14.990	14.624	1.970
19p	4.837	4.528	5.953	5.106	0.750
28p	5.628	7.218	5.878	6.241	0.855
29p	9.488	8.995	9.671	9.385	0.350
30p	14.404	14.159	14.905	14.489	0.380
34p	10.841	10.410	11.180	10.810	0.386
35p	9.525	8.054	9.664	9.081	0.892
36p	15.379	15.691	15.963	15.678	0.292
42p	6.115	5.668	7.469	6.417	0.938
45p	6.585	9.669	7.634	7.963	1.568
46p	9.189	10.403	11.716	10.436	1.264
47p	13.376	15.174	14.530	14.360	0.911
49p	5.381	5.822	7.506	6.236	1.121
50p	7.406	6.682	7.492	7.193	0.445

Appendix 13. Readings for group C (non-FGSRD) after 28 days (µm)

CD slabs Days 28	Reading 1	Reading 2	Reading 3	Mean	Stand Dev
1p	43.324	42.894	42.985	43.068	0.227
2p	41.974	39.589	39.233	40.265	1.490
5p	38.068	40.25	39.142	39.153	1.091
6p	41.621	40.794	41.486	41.300	0.444
7p	34.098	35.653	34.953	34.901	0.779
11p	40.895	41.658	41.198	41.250	0.384
12p	42.852	42.572	42.301	42.575	0.276
14p	41.501	41.354	41.902	41.586	0.284
15p	34.144	34.058	33.769	33.990	0.196
21p	54.525	56.515	53.729	54.923	1.435
22p	40.557	42.625	40.727	41.303	1.148
23p	31.539	34.705	32.265	32.836	1.659
24p	40.324	43.133	43.187	42.215	1.638
25p	27.624	28.412	33.996	30.011	3.474
31p	48.381	55.921	53.196	52.499	3.818
37p	37.677	38.218	38.356	38.084	0.359
38p	41.178	40.425	39.357	40.320	0.915
39p	41.358	41.939	41.993	41.763	0.352
40p	51.237	44.304	50.655	48.732	3.846
41p	57.606	46.745	57.85	54.067	6.342

Appendix 14. Readings for group D (FGSRD) after 28 days (µm)

DD slabs Days 28	Reading **1**	Reading **2**	Reading **3**	M**ean**	**Stand Dev**
3p	28.553	28.046	25.293	27.297	1.754
4p	36.009	34.612	33.903	34.841	1.072
8p	47.432	49.473	48.019	48.308	1.051
9p	24.018	25.845	24.856	24.906	0.915
13p	24.115	24.454	35.486	28.018	6.469
17p	21.614	24.982	26.599	24.398	2.543
18p	37.399	33.284	36.732	35.805	2.209
19p	25.45	22.614	23.621	23.895	1.438
28p	30.218	27.643	26.109	27.990	2.076
29p	32.184	31.468	32.17	31.941	0.409
30p	39.142	40.933	35.983	38.686	2.506
34p	36.973	32.09	37.045	35.369	2.840
35p	28.772	30.011	29.105	29.296	0.641
36p	42.508	41.75	38.233	40.830	2.281
42p	47.71	48.077	38.2	44.662	5.600
45p	20.136	23.086	24.778	22.667	2.349
46p	33.383	30.318	36.933	33.545	3.310
47p	36.664	35.804	35.662	36.043	0.542
49p	27.092	28.12	27.337	27.516	0.537
50p	28.277	23.063	27.779	26.373	2.877

Appendix 15. Intra-examiner correlation readings (µm)

ID	Day	Groups	Reading A	Reading B	Day	Reading A	Reading B
c2a	14	A	19.099	19.133	28	46.080	44.221
5a	14	A	13.895	15.158	28	32.989	31.885
A3a	14	A	14.006	15.026	28	41.318	40.994
7a	14	A	13.741	13.899	28	41.089	40.371
11a	14	A	11.323	11.908	28	30.601	30.493
24a	14	B	17.864	18.839	28	43.664	43.646
33a	14	B	16.309	16.606	28	41.113	40.558
13a	14	B	12.113	12.291	28	30.289	29.810
16a	14	B	9.559	9.509	28	26.456	25.080
29a	14	B	12.723	13.080	28	31.206	32.048
15p	14	C	9.101	8.894	28	33.990	33.167
7p	14	C	11.499	11.394	28	34.901	34.768
39p	14	C	14.684	14.351	28	41.763	41.442
25p	14	C	10.619	9.390	28	30.011	29.411
1p	14	C	15.553	15.594	28	43.068	42.690
50p	14	D	7.193	7.304	28	26.373	25.486
13p	14	D	8.075	8.661	28	28.018	27.229
3p	14	D	9.308	9.727	28	27.297	25.973
30p	14	D	14.489	14.041	28	38.686	38.555
45p	14	D	7.963	7.888	28	22.667	22.718

Appendix 16. Results after the F analysis of the devices (ppmF)

Days	FGSRD A	FGSRD B	FGSRD C	Placebo A-C
1	0.41	0.17	0.12	0
2	3.37	6.05	2.97	0
3	0.29	0.43	0.14	0
4	0.02	0.09	0.02	0
5	0.02	0.14	0.02	0
6	0.05	0.17	0.03	0
7	2.48	0.17	0.1	0
8	0.56	0.2	0.08	0
9	0.44	0.65	0.16	0
10	4.57	0.82	0.18	0
11	1.21	1.07	0.22	0
12	1.23	0.87	0.31	0
13	1.68	2	0.37	0
14	2.1	2.16	0.33	0
15	2.12	1.3	0.55	0
16	6.06	1.58	0.57	0
17	6.15	3.5	0.56	0
18	4.25	6.87	1.63	0
19	4.53	3.25	0.78	0
20	3.94	3.88	0.95	0
21	3.27	3.18	0.77	0
22	4.02	4.35	0.99	0
23	3.91	4.68	1.03	0
24	5.1	4.7	1.06	0
25	5.49	3.9	1.04	0
26	4.96	4.44	1.19	0
27	5.94	6.97	1.22	0
28	12	6.96	1.55	0
mean	3.32	2.08	0.555	0
standard deviation	2.68	2.31	0.66	0