Fluoride Release and Uptake from Resin-Modified Glass Ionomer Cements used for Orthodontic Purposes - an *in vivo* investigation

by

Chor Kwan Chung, B. D. S.

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This research was conducted at
Glasgow Dental Hospital and School,
378 Sauchiehall Street,
Glasgow G2 3JZ

in collaboration with the
Lambert and Hunter
Dental Practice
72, Cadzow Street,
Hamilton ML 3 6 DS

ProQuest LLC.
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106 – 1346

ABSTRACT

AIMS: The cariostatic ability of two resin-modified glass ionomer cements (Vitremer and Dyract) was compared to that of a conventional adhesive resin (Right-On) in a half-mouth orthodontic bonding trial. The local and systemic uptake of fluoride from the two resin-modified glass ionomer cements were also compared to those from the conventional adhesive resin.

MATERIALS AND METHODS: One of the resin-modified cements was allocated to each of 26 patients (mean age 13.4 years) at random in a half-mouth orthodontic bonding trial. Only non-carious premolar teeth scheduled for orthodontic extraction were included in the study. Each patient used a non-fluoride toothpaste for four weeks prior to bracket bonding and for the four week trial period. To assess decalcification, colour transparencies of each extracted tooth (96 in total) were coded and randomly arranged and projected onto a screen in a darkened room. Each transparency was scored by two examiners independently under identical conditions, two weeks apart, using a modified caries index (0='no white spot'; 1='frank white spot'; 2='cavitation'). Salivary and urinary fluoride concentrations were measured pre-bonding and 4 weeks post-bonding. Plaque samples around bonded brackets on premolars were analysed also for fluoride concentration on the two occasions.

RESULTS: 2% of premolars bonded with the fluoride-releasing materials in contrast to 19% of premolars bonded with the nonfluoride-releasing material developed early carious lesions or cavitation after 4 weeks *in vivo*. In relation to the degree of decalcification observed, a significant difference was found between Vitremer and Right-On ($p=0.033$), but the difference between Dyract and Right-On was not significant ($p=0.179$). Neither Vitremer nor Dyract altered salivary fluoride concentration significantly 4 weeks post-bonding (0.012ppm and 0.009ppm respectively; $p>0.05$ t-test). The difference in urinary fluoride concentration was not statistically significant for both test

materials (0.051ppm Vitremer; 0.048ppm Dyract; $p>0.05$ t-test). Plaque fluoride concentration increased significantly around premolars bonded with Vitremer (upper arch $p=0.032$; lower arch $p=0.012$; t-test). A slight but insignificant increase in plaque fluoride concentration was observed around premolars bonded with Dyract.

CONCLUSIONS: 1. The cariostatic ability of the resin-modified glass ionomer cement, Vitremer, was superior to that of the nonfluoride-releasing cement Right-On, but there was no significant difference between the cariostatic ability of Dyract versus Right-On. 2. Fluoride release from the resin-modified glass ionomers (Vitremer and Dyract) is likely to exert a local and not a systemic effect.

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Acknowledgments

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DECLARATION

This thesis is the original work of the author

CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

1.1 INTRODUCTION AND AIMS

Demineralisation or white spot formation has long been recognised as a problem during fixed appliance orthodontic treatment (Meyers, 1952). Retention of plaque and reduced oral hygiene efficiency have been identified as the principle aetiological factors (Zachrisson and Zachrisson, 1971). Between 34-50% of patients experience demineralisation (Geiger *et al.*, 1988; Gorelick *et al.*, 1982) and this can be noticeable around fixed orthodontic attachments after only one month into treatment (O'Reilly and Featherstone, 1987). To prevent demineralisation, careful oral hygiene and fluoride preventive programmes have been recommended by many clinicians but compliance with these regimes has been poor (Geiger *et al.*, 1988).

In an effort to overcome the difficulties with these programmes, interest has been focused on developing fluoride-releasing materials which exert a local cariostatic effect at the sites where decalcification is likely during fixed appliance orthodontic treatment. A few clinical trials have studied the ability of fluoride-releasing composite resins to prevent demineralisation, but results remain equivocal (Sonis and Snell, 1989; Mitchell,1992).

More recently glass ionomer cements have been used for orthodontic bonding and they have important potential cariostatic properties. They leach fluoride over long periods into adjacent enamel and have a capacity to absorb fluoride from fluoride supplements, thus acting as a rechargeable slow release fluoride

sump (Hatibovic-Kofman and Koch, 1991). In addition some of the released fluoride can also be distributed into whole saliva, subsequently swallowed and taken up systemically (Hallgren,1990). Using conventional glass ionomer cements for bracket bonding, a significant reduction in white spot lesions has been reported *in vivo* (Marcusson *et al.*, 1993). However, the variable bond strength of these cements precludes their routine use as a bonding material in clinical orthodontic practice (Cook, 1990; Fricker, 1992).

In an effort to improve the bond strength of conventional glass ionomer cements while retaining their fluoride releasing capacity, a new group of materials, resin-modified glass ionomers or 'compomers', have been marketed recently. These are composed of composite and glass ionomer particles and preliminary reports appear to indicate that they have reliable bond strengths and perform as well clinically as composite resins (Fricker, 1994; Silverman, 1995). Compomers, have been shown to release fluoride *in vitro* (Chadwick and Gordon, 1995; Lee and Kim, 1995) but as yet there is only anecdotal evidence relating to their ability to prevent decalcification *in vivo* (Silverman *et al.*, 1995).

The **aims** of this study were twofold:

1. To compare the cariostatic ability of two resin-modified glass ionomer cements (Vitremer and Dyract) to that of a conventional adhesive resin (Right-On).
2. To compare the local and systemic uptake of fluoride from two resin-modified glass ionomer cements (Vitremer and Dyract) with those from a conventional adhesive resin (Right-On) by quantifying the fluoride concentration in plaque, saliva, and urine.

From these aims this thesis attempted to address the following two **null hypotheses**:

1. There is no significant difference in decalcification observed with the resin-modified glass ionomer cements (Vitremer or Dyract) compared to that observed with the conventional adhesive resin (Right-On).
2. There is no significant difference in the local and systemic uptake of fluoride from the two resin-modified glass ionomer cements (Vitremer and Dyract) when compared with those from the conventional adhesive resin (Right-On).

LITERATURE REVIEW

1.2 DENTAL CARIES

1.2.1 INTRODUCTION

Dental caries or tooth decay is initiated by microbial activity at the tooth surface with subsequent loss of mineral and structural integrity. It is one of the most common oral diseases in both the industrialised and third world countries often resulting in pain or discomfort and possibly tooth loss if the destruction is beyond repair.

At a molecular level, caries is a dynamic process of mineral exchange between dental hard tissues and their immediate fluid environment. Early enamel lesions or white spot lesions, are reversible through remineralisation (Featherstone *et al.*, 1982) but the destruction brought about by the caries process may become progressive if there is a net loss of mineral.

Orthodontic treatment, with fixed appliances, is often associated with a higher rate of dental caries. The prevalence, severity and distribution of dental caries related to orthodontic treatment are discussed in section 1.5. To tackle the caries problem, it is important to understand first the aetiological factors, the caries process and the role of fluoride in caries prevention.

1.2.2 EARLY AND ALTERNATIVE VIEWS OF CARIES AETIOLOGY

There were many theories, sometimes speculations, put forward to explain the cause of dental decay. Among these, the earliest was recorded in an ancient Sumerian text which dates back to 5000 years BC. It was believed that the "tooth worm" caused tooth decay and the misery of tooth ache. The ancient

Chinese appeared to be in favour of this "tooth worm" theory also, as Chinese characters on oracle bones in the Shang dynasty, around 1000 BC, were found to support this explanation (Newburn, 1983).

From the end of the 18th century until the middle of the 19th century, the common view was that caries originated from within the tooth itself (Nikiforuk, 1985). This proposed "Vital Theory" was challenged by Parmly (1819), who believed that a chemical substance was responsible for dissolving the tooth and the formation of cavities. This view was supported by Robertson (1835) who identified this chemical substance as an acid formed by the fermentation of food particles around the teeth. On the other hand, Eardl (1843) suggested that it was a parasite on the tooth surface that caused caries. The mechanism of tooth destruction was not mentioned, but the parasitic theory was based on the fact that micro-organisms had been isolated by Van Leeuwenhoek (1632 - 1723) from caries substance.

The dissolution of tooth mineral after exposure to acids was observed by many including Magitot (1867) and Williams (1898). Miller (1883), through his own investigations and the inference of others work, amalgamated the chemical and parasitic theories and concluded that dental caries was caused by acids produced by micro-organisms of the mouth. He considered that acid was produced by the fermentation of impacted food debris by bacteria in saliva. Black (1898) proposed that it was acid produced *in situ* that caused tooth destruction. This view was supported by Williams (1898), who observed dental plaque on the enamel surface and regarded that acid produced inside dental plaque had a more localising effect.

Half a century latter, Gottlieb (1944) proposed his "Proteolytic Theory" which suggested that the organic element of the enamel is first attacked by

proteolytic bacteria, and that the inorganic component is then subsequently lost either by acid dissolution (Frisbie and Nuckolls, 1944) or by the mechanical loss of physically unbounded prisms (Pincus, 1949). This theory was widely criticised since enamel actually contains a very small amount of organic component. Furthermore, those parts of enamel with a higher organic content, such as tufts and lamellae, do not show greater susceptibility to decay.

The "Proteolysis-Chelation Theory" was put forward by Schatz and Martin (1962). They proposed that products of proteolysis of tooth substance, acquired pellicle and food may act as chelating agents, thereby releasing mineral ions from enamel. However, the amount of chelating agent released by proteolytic degradation of the organic phase of enamel is likely to be negligible (Jenkins, 1978). Perhaps it may play a minor role in the caries process for a period after plaque pH returns to neutrality following an acid pulse (Silverstone *et al.*, 1981).

1.2.3 CURRENT THEORIES OF CARIES AETIOLOGY

Dental caries is a multifactorial disease . The disease process consists of many aetiological and modifying factors. The initiation of a lesion is due to the concurrent presence and interaction of four primary factors: the host, the microflora , the substrate and time (Newburn, 1983). Secondary factors modify the initiation and the progression of the disease. These include the type and pattern of substrate used, salivary composition and flow, site and mineral status of the tooth and the cariogenic potential of the microflora in the dental plaque. Each of these aetiological factors will be examined in the following sections.

1.2.4 MICROFLORA

Bacteria which inhabit the mouth constitute a community of many different species. Those which can adhere effectively to the tooth surface, produce appreciable amounts of acids and secrete extracellular polysaccharides which are likely to be cariogenic. Among these, Streptococcus mutans (S. mutans) has been shown to be most abundant in human subjects (Marsh & Martin, 1984). The first conclusive evidence that demonstrated the bacterial role in caries initiation was produced by Orland *et al.*(1954). He demonstrated that completely germ-free rats of a caries-susceptible line failed to develop caries even when fed a cariogenic, high-sucrose diet. By contrast, when bacteria were implanted into the mouths of these germ-free animals, caries was produced. Cross-sectional surveys (Krasse *et al.*, 1968; Loesche *et al.*, 1975) on human populations have indicated an association between the presence of S. mutans and the degree of caries experience.

1.2.5 BACTERIAL SUBSTRATE

Several epidemiological studies have shown conclusively that fermentable carbohydrates, especially sucrose, predispose to caries. Toverud (1957) carried out a longitudinal study on the influence of war and post-war conditions on the teeth of Norwegian school children. His work showed clearly a lower caries prevalence in Norwegian children with a reduced intake of carbohydrates.

Lilienthal (1953) and Sullivan & Harris (1958) compared the incidence of caries in Hopewood House, where the children were mainly on a vegetarian diet, with that of the local non-institutionalised population of the same age. At age 13, 53% of the children in Hopewood House were caries free compared with only 0.4% of the local population whose diet was partly made up of

fermentable carbohydrates. This demonstrated clearly the importance of dietary control to dental health.

The Vipeholm study (Gustafsson *et al.*, 1954), determined the effect of different levels of carbohydrate intake on caries activity in 436 individuals observed for five years. The quantity, form (i.e. stickness) and frequency of sugar consumption correlated with the caries rate. Caries prevalence varied from person to person under identical test conditions, highlighting the importance of interaction of the caries determinants .

Xylitol and other non-fermentable carbohydrates have been found to produce less acid in the oral enviroment (Vadeboncoeur *et al.*,1983) and are considered to be less cariogenic.

1.2.6 THE TOOTH

The prevalence of caries lesions varies from tooth to tooth around the same mouth; molar teeth have the highest incidence of tooth decay (Carlos & Gittleschon, 1965; Todd & Lander, 1991). Occlusal fissures, interproximal contact areas and gingival margins encourage food retention and are, therefore, more susceptible to caries attack (Cole and Eastoe, 1988). On the other hand, free smooth surfaces of the teeth such as the buccal and lingual surfaces are more caries resistant (Backer Dirks, 1965).

The observed caries reduction on the buccal and lingual surfaces was thought to result from incorporating fluoride into the enamel mineral during tooth formation, thus reducing enamel solubility. Many studies (Schamschula *et al.*, 1979; Keene *et al.*,1973; Shern *et al.*,1977) have failed to demonstrate a correlation of enamel fluoride content with caries experience. Only one study to date has demonstrated a positive correlation between these two parameters

(DePaola *et al.*, 1975). Groeneveld (1985) suggested that fluoride inhibits primarily caries *progression* and has a relatively small effect on *caries incidence.*

In fact, fluoride imparts its cariostatic effect by different mechansims, depending upon the amount of fluoride present at the enamel-plaque interface. The importance of the systemic and topical effect of fluoride in caries prevention is discussed in section 1.4.

Both carbonate and magnesium content in the tooth substance have been implicated also in the caries process, although very little is known about their importance. Little and Brudevold (1958) suggested that the carbonate content of enamel was a determining factor for enamel caries susceptibility. Whether these ions render the enamel more soluble needs further investigation.

1.2.7 THE FORMATION AND COMPOSITION OF DENTAL PLAQUE

When a clean tooth surface is exposed to the oral enviroment, it becomes coated rapidly with an amorphous organic layer derived principally from salivary components. This acquired pellicle is composed mainly of undegraded salivary glycoproteins. When bacteria and their products begin to accumulate on the pellicle, it is defined as plaque.

The rate of formation of dental plaque varies with individuals. The amount and type of plaque also vary in different physical environments. Crowded teeth, overhanging margins and rough surfaces of restorations as well as orthodontic appliances interfere with oral hygiene procedures and encourage plaque formation (Cole and Eastoe, 1988).

Dietary factors also influence the formation of dental plaque. A sucrose rich diet has been shown to increase the microbial density and the numbers of Streptococcus mutans and Lactobacilli (Staat *et al.*, 1973). Carbohydrate metabolism by bacteria in dental plaque results in the production of a variety of organic acids causing the pH to drop (Geddes, 1972). The subsequent tooth destruction is basic to the pathogenesis of caries.

1.2.8 ROLE OF SALIVA IN THE CARIES PROCESS

It is widely believed that saliva through a combination of chemical, physical and biological actions exerts a protective mechanism against cariogenic micro-organisms. The normal physiological functions of saliva include: mechanical cleaning of the oral cavity, buffering, regulation of calcium/phosphate equilibrium, modulation of the oral flora, lubrication and hydration, antacid and neutralization of deleterious materials and digestion.

Sailvary flow

There is a great variation in the rate of flow between individuals (Becks, 1939). Although the average resting rate is about 19 ml per hour (Becks and Wainwright, 1943), salivary flow rate varies throughout the day with periods of high flow rate in the early afternoon and evening (Dawes, 1975) and a reduced rate during sleeping hours (Schneyer *et al.*, 1956). Salivary flow is increased during eating (Jenkins, 1978).

It is well recognised that catastrophic loss of salivary function leads to an increased risk of dental caries (Fox *et al.*, 1985). Xerostomia may occur following irradiation of the salivary glands, obstruction of the salivary ducts, in individuals with Sjorgren's syndrome and as a side effect of certain drug therapy. The deterioration of oral health may be extremely rapid and dramatic with characteristic production of widespread cervical lesions (Brown, 1975). A

high salivary flow rate is known to be related to greater buffering power, but it is less clear whether there is a correlation with caries experience.

Buffering power

This is the power to resist changes of pH when acid or alkali are added (Jenkins,1978). Salivary buffers consist of bicarbonates, phosphates and proteins. They are effective over different parts of the pH range. Lilienthal (1953) concluded that bicarbonate is the most important buffer in saliva and phosphate plays some part. Bicarbonates release the weak carbonic acid when an acid is added and since carbonic acid decomposes rapidly into water and carbon dioxide, which leaves the solution, the result is the complete removal of acid. It is the ability to bind hydrogen ions that enables the buffers to raise the pH of the oral enviroment after an acid attack.

calcium/phosphate equilibrium

The saturation of saliva by calcium and phosphate ions is believed to be important in the dynamics of dental caries. The concentration of these ions influences the rate at which apatite dissolves, by the law of mass action. It is also clear that the concentration of ions needed to prevent apatite from dissolving depends on the pH. The pH at which any particular saliva ceases to be saturated is referred to as the "critical pH" and below this value, the inorganic material of teeth may dissolve (Fosdick & Strake, 1939). In unstimulated saliva, the pH and concentration of calcium and phosphate ions are higher and it is regarded as supersaturated with respect to hydroxyapatite (Gron, 1973).

1.2.9 OTHER MODIFYING FACTORS

It seems that if cariogenic organisms develop in bacterial communities (dental plaque) in response to the enviroment, then modification of the enviroment

could reverse or disrupt the process. Physical disruption of plaque, such as brushing and flossing, results in the dispersion of plaque acid. In fact, Holmen *et al.* (1988) have shown that mechanical disturbance of plaque prevents demineralisation. Incorporation of enzymes, like dextranase, into toothpaste can disrupt plaque by lysing its matrix, thereby modifying the rate of diffusion of acid through plaque (Mandel and Kleinberg, 1986). Several other chemicals including sodium bicarbonate and ammonium salts have been incorporated into toothpastes and mouth rinses to increase plaque pH. Sodium bicarbonate incorporated in chewing gum has been found to increase pH in human interproximal plaque (Igarashi *et al.*, 1988).

Mineralising solutions in the form of mouth rinses have been used to increase the level of plaque mineral and affect the balance of demineralisation and remineralisation in favour of the latter (Pearce and Nelson, 1988). Some species of oral bacteria produce urease which converts urea to ammonia (Macpherson and Dawes, 1991), hence neutralising acid and increasing the pH in plaque. Little is known, however, about how to promote this activity effectively within the ecosystem in plaque (Gallagher *et al.*, 1984). Anti-bacterial agents such as chlorhexidine have had very limited success with caries reduction (Lundstrom *et al.*, 1987). The role which fluoride plays in affecting bacterial metabolism and plaque ecology will be discussed in section 1.4.

1.2.10 SUMMARY

Dental caries is a multi-factorial disease. It is only initiated with the concurrent presence and interaction of host, microflora and substrate. Modifications of the primary and secondary factors may reverse or disrupt the caries process.

1.3 THE CARIES PROCESS IN ENAMEL

1.3.1 INTRODUCTION

The early stages of enamel dissolution occur at a submicroscopic level. It only becomes apparent clinically when the loss of mineral is visible as a white spot. This appearance is caused by an optical phenomenon owing to subsurface tissue loss .

1.3.2 STRUCTURE AND COMPOSITION OF ENAMEL

Normal enamel appears hard and shiny with a glass-like appearance. The apparent yellow-white colour of teeth is a result of the underlying dentine "showing through" the enamel layer.

Enamel is the most highly mineralised tissue in the human body. It consists of 96% mineral and 4% organic material and water. The inorganic content of enamel is made up of crystalline calcium phosphate known as hydroxyapatite.

The primary ionic constituents are calcium ions, phosphate ions and hydroxide ions. Carbonate ions, biphosphate ions and trace elements take up some positions in the crystalline lattice by replacing the primary elements. Some of these ions, particularly carbonate, are relatively easily released from enamel during demineralisation, and positions in the lattice where carbonate ions are present are believed to be particularly vulnerable to the effects of acids (Featherstone *et al.*, 1979). Other ions, such as fluoride, may be included in the apatite and are only released when the crystals dissolve (Nikiforuk, 1985). The organic materials are largely proteinaceous and contain some polysaccharides (ten Cate, 1989).

The crystals in the enamel are arranged in an orderly fashion forming rods and interrod enamel. The crystal arrangement is slightly looser in the rod periphery compared with the rod and interrod enamel. The intercrystalline spaces are filled with water and organic material. These form a fine network of potential diffusion pathways which are referred to as micropores in enamel.

1.3.3 DEMINERALISATION IN ENAMEL

In the presence of fermentable carbohydrate, especially simple saccharides, organic acids are produced by micro-organisms within dental plaque (Geddes, 1991).

In the plaque-enamel interface, where plaque fluid is in contact with the enamel surface, an incipient dissolution of the enamel surface occurs. This produces a surface softening effect (Ogaard *et al.*, 1986) with different morphological changes such as enlargement of the interprismatic spaces, development of micro-fissures and formation of microchannels (Thylstrup *et al.*,1982).

Organic acids diffuse into enamel predominantly in the non-ionised form. Once within the enamel, protonation occurs at specific sites on the apatite lattice. The degree and extent of protonation depend on the local pH, concentration of organic acids and degree of saturation of enamel mineral.

Different organic acids react at different rates. Lactic acid dissociates more readily than acetic acid, although it will not diffuse into enamel as fast (Featherstone & Rodgers, 1981). The higher the rate of protonation, the faster the rate of diffusion of ionic calcium and phosphate out of enamel, and hence the faster the rate of lesion formation.

Initial dissolution is usually followed by the precipitation of less soluble solid phases of dicalcium phosphate dihydrate (DCPD: $CaHPO_4.2H_2O$) and fluoridated hydroxyapatite (FHA), where fluoride is derived from the intact enamel mineral. Within the outer enamel layer, there is an equilibrium between enamel mineral (i.e. hydroxyapatite, HA), DCPD, FHA, and the acidic solution within the enamel pores. As the acidic solution diffuses from the pores of the outer enamel surface into inner enamel regions, it dissolves the underlying enamel. With the increase in concentration gradient of enamel mineral in the fluid, these ions diffuse back into the surface layer and induce the precipitation of mineral phases in this region. A portion of these ions diffuse out of the enamel surface into the oral enviroment. While the lesion progresses, ions are pumped from the inner enamel region into the surface layer and from the surface layer into the surrounding enviroment. The surface layer appears unaltered because it is being continuously regenerated (Margolis and Moreno, 1985) although a subsurface lesion has formed.

Nishimura (1926) was the first to describe the phenomenon of zonation in the early caries lesion. The zones in the lesion can be expressed in terms of spaces present or, conversely, tissue loss. The first zone of histologic change is called the translucent zone. Here, there is an absence of structural rod outlines and a tenfold increase in the amount of space compared to normal, unaffected enamel. Progressing into the lesion, the next zone is the dark zone, so called because of its appearance in polarized light microscopy. This zone exhibits a further increase in the volume of spaces. It has been shown that remineralisation occurs in the dark zone (Silverstone,1977). Finally, the core of the rods is involved, producing a zone of maximum tissue destruction termed the body of the lesion. The outermost layer of enamel, above the lesion, remains relatively intact and appears radiopaque on radiographs.

1.3.4 REMINERALISATION IN ENAMEL

"Remineralisation" was originally used to describe the complete repair of a demineralised tooth surface. This was observed clinically as the disappearance of white spot lesions (Backer Dirks, 1966) and by the chalky appearance of etched enamel.

"Remineralisation" is now commonly used to describe the process of mineral deposition (Larsen, 1973). Remineralisation occurs not only during periods of neutral pH, when minerals precipitate from the oral fluids in the enamel defects, but also during caries development.

A prerequisite for enamel lesion remineralisation is the presence of partly demineralised crystallites which act as nuclei for mineral deposition. Enamel lesion remineralisation is limited by the diffusion of ions from the external fluids (Featherstone *et al.*, 1983; Ten Cate and Arends, 1977). As a result, precipitation may occur preferentially in the surface layer unless there is an inhibition of crystal growth in this area because of the presence of precipitation inhibitors (e.g. salivary proteins). The mechanism by which remineralisation is promoted by the presence of fluoride ions will be discussed in the next section.

1.3.5 SUMMARY

Enamel caries is a dynamic process. The net movement of tooth mineral away from and into the lesion constitutes the dynamic equilibrium of demineralisation and remineralisation respectively. The local pH, concentration gradients of enamel minerals and the concentration of organic acids determine the direction and rate of flow of the ions. The early enamel lesion is reversible by remineralisation through deposition of ions from the surrounding oral fluid. There is substantial evidence that the initial step in development of a caries

lesion is mineral loss at the very surface of the enamel; such surface softened lesions may in the presence of fluoride in the oral fluid develop into subsurface lesions. These white spot lesions may regress or even disappear as a result of remineralisation.

1.4 FLUORIDE AND ENAMEL CARIES

1.4.1 INTRODUCTION

Better oral hygiene practices, reduction in the frequency of sugar intake in particular, and easy access to dental care services are all known to have strong associations with the decline in caries experience. Fluoride is still regarded, however , as the most important single factor in combating caries.

It is now believed that the effectiveness of fluoride in caries prevention is due to its presence in the aqueous phase during enamel dissolution and that it acts in three different ways:

(I) Inhibition of demineralisation

(II) Enhancement of remineralisation

(III) Effects on bacteria

Contrary to previous beliefs, Newesely (1972) stated that the reduction in enamel solubility by pre-eruptive incorporation of fluoride is small. It is unlikely that fluoridated hydroxyapatite plays an important role in the observed caries reduction (Larsen, 1973; Fejerskov *et al.*, 1981)

1.4.2 HISTORICAL ACCOUNT

The effect of fluoride on enamel was first noticed in Colorado Springs, U.S.A., where Mckay (1901) observed and documented the condition of

"mottling". He found that this condition, known as "Colorado brown stain", only affected people if they had been brought up in this area during the period of enamel formation. The group with this "mottled" enamel were found to be associated with a lower prevalence of caries.

At around the same time, independently, Eager (1902) reported the "mottling" condition of enamel in Naples, Italy. He suggested that this was related to the local water supply in Naples . It was noted also that the incidence of such "mottling" decreased when the water supply was changed.

Further reports by Mckay and Black (1916) confirmed that enamel mottling had a geographic distribution related to the local water supply and caries prevalence was less in areas where "mottling" was endemic. McKay (1925) later observed the absence of enamel mottling in children born after the water supplies to the towns of Britton and Oakley were altered. In the United Kingdom, Ainsworth (1933) showed that "mottling" was due to high levels of fluoride and caries experience in a fluoride area was lower than average .

Dean's report (1938), commissioned by the Public Health Department in the United States, confirmed that the concentration of fluoride in water was correlated to the clinical severity of fluorosed enamel. The addition of up to 1 ppm fluoride to water gave a caries reduction of 60% with no significant fluorosis.

Since dental fluorosis is caused by pre-eruptive consumption of fluoride, it is not surprising that the cariostatic properties of fluoride were throught to be due to the incorporation of fluoride into the enamel.

1.4.3 FLUORIDE METABOLISM

The gastric absorption, tissue distribution, and renal excretion of fluoride are influenced by the magnitude and direction of the pH gradient between adjacent body fluid compartments (Whitford, 1990). The kidney is the major route for the removal of fluoride from the body, roughly 50% of fluoride absorbed from the gastrointestinal tract of adults being excreted in the urine. Uptake by bone and other calcified tissues is the second major mechanism by which fluoride is cleared from plasma and the other body fluids. The clearance rate of fluoride from plasma is essentially equal to the sum of the renal and skeletal clearance rates (Ekstrand *et al.*, 1980).

Factors which alter urinary pH may affect profoundly the amount of fluoride reabsorbed. Such factors include the composition of the diet, certain metabolic or respiratory diseases, certain drugs and the altitude of residence. When the urine is relatively alkaline, nearly all of the fluoride exists in ionic form, which because of its charge and size, remains within the tubule to be excreted.

After ingestion of a small dose of fluoride, measurable increases in plasma occur within the first few minutes. The peak plasma concentration, which is followed by a rapid decline, typically occurs at about 30-45 mins. The urinary ionic fluoride level, which is very close to the plasma concentration, is widely regarded as one of the best markers of fluoride intake systemically.

1.4.4 SYSTEMIC EFFECT OF FLUORIDE IN CARIES PREVENTION

It was believed widely that fluoride reduces enamel caries by incorporating fluoride ions into the crystal lattice. Replacement of hydroxyl groups with the smaller fluoride ion results in a more stable apatitic structure which in turn reduces enamel solubility in acid (Brown *et al.*, 1977). The amount of

fluorapatite present in enamel is, however, relatively small. The degree of fluoride substitution in enamel has been found to be 8% and 5.6% from fluoridated and non-fluoridated areas respectively (Aasenden *et al.*, 1971). Such levels of fluoride incorporation provide limited caries protection by reducing enamel solubility. Surface absorbed fluoride has been found also to be more effective at reducing enamel mineral dissolution than fluoride incorporated into the hydroxyapatite lattice (Wong *et al.*, 1987). In fact, the pre-eruptive effect would be lost unless fluoride is also consumed post-eruptively (Backer Dirks *et al.*, 1961; Marthaler,1967).

Furthermore, studies by Melberg *et al.* (1985) and Retief *et al.* (1987) failed to establish a strong link between enamel fluoride and caries susceptibility. Fejerskov *et al.* (1981) and Hardwick *et al.* (1982) have concluded that the cariostatic effect provided through water fluoridation results predominantly from a topical effect.

The pre-eruptive anticariogenic value of fluoride is unclear. It is believed that a similar reduction can be achieved by the use of topical fluoride alone. Several studies in non-fluoridated areas where topical fluoride supplements were used have shown caries reductions comparable in magnitude to those observed in fluoridated areas (Groeneveld *et al.*,1990).

1.4.5 TOPICAL EFFECT OF FLUORIDE IN CARIES PREVENTION

Fluoride is present in oral fluids and accumulates in dental plaque. The concentration of fluoride in plaque is about 100 times greater than in saliva. The sources of plaque fluoride include saliva, gingival fluid, certain foods and liquids in the diet, topically applied therapeutic agents and possibly demineralising tooth hard tissues.

Several studies suggest that much of the fluoride in plaque is held in compounds which are in dynamic equilibrium with the plaque fluid and may release fluoride for a long period of time after administration. These reservoirs of loosely bound fluoride in plaque may be released under mild acidic conditions (Ophaug *et al*; 1987). In addition, fluoride is known to have anti-bacterial properties, but there is no consensus of opinion over the extent to which these properties contribute to fluoride's anticariogenic effect.

It is known that fluoride can affect plaque flora in the following ways:

1. A high concentration of fluoride inhibits growth of Streptococcus mutans species and other micro-organisms (Hamilton and Bowden, 1988).
2. Fluoride reduces acid tolerance of S. mutans and other micro-organisms, leading to a less acidogenic plaque flora (Marquis,1990).
3. Fluoride inhibits enzymic reactions and leads to a slower rate of acid production (Geddes and McNee, 1982).
4. Fluoride alters carbohydrate metabolism, reduces production of extracellular polysaccharides and hence adhesion to the enamel surface (Jenkins *et al.*, 1977; Dawes *et al.*, 1989).
5. Fluoride inhibits protein translocating ATPase, thus, reducing growth of bacteria (Sutton *et al.*, 1987).

ten Cate and Duijsters (1983) have reported that low concentrations of fluoride (as little as 0.05ppm) reduce the rate of demineralisation *in vitro*, especially at low pH values. Margolis *et al.* (1986) recorded such a phenomenon also with a fluoride concentration of 0.024ppm. In an *in vivo* situation, any elevation of salivary fluoride concentration may reduce enamel demineralisation, probably by increasing the degree of saturation of saliva with respect to tooth mineral, thereby promoting remineralisation of early caries lesions (Dawes and Weatherell, 1990). When the rate of deposition of fluorapatite or

remineralisation exceeds the rate of transportation of ions out of the enamel surface, enamel demineralisation will be inhibited effectively.

At higher concentrations of fluoride, calcium fluoride is formed on the tooth surface. Although this mineral is sparingly soluble in saliva, the process of tooth mineral dissolution is retarded for periods of up to a week or longer by surface deposition of salivary phosphate and pyrophosphate. Some fluoride may enter dental plaque and subsequently diffuse out.

Calcium fluoride will release fluoride during the caries challenge due to a reduced concentration of $(HPO_4)^{2-}$ at acid pH (Rolla, 1988). Normally, the fluoride released from calcium fluoride during the caries challenge is subsequently built into hydroxyfluorapatite through dissolution/re-precipitation reactions. Hence, fluoride is effectively being 'stored' in the mouth on the enamel surface and then released slowly.

Overall, the result is the slowing down and reduction in pH decrease .It is still impossible to apportion the anticaries effect of fluoride between plaque mediated mechanisms and hard-tissue protection or repair (Bowen, 1990).

1.4.6 CARIES PREVENTION METHODS USING FLUORIDE

Fluoridation of water supplies, where possible, remains the most effective public health measure for prevention and subclinical treatment of dental caries. This is simply attributable to the fact that water is a dietary component required and used by everyone.

However, not all domestic supplies world-wide contain an effective level of fluoride. In a developed country, this may be due to government health policy and political reasons that make fluoridation of drinking water impossible. In developing countries or in rural areas, there may be difficulties in providing a reliable and controllable water supply due to economic and geographical reasons.

There are, nonetheless, many alternatives to fluoridating the water supply which are as follows:-

(I) DIETARY SUPPLEMENT (TABLETS AND DROPS)

Caries prevention of the order of 39-80% has been reported by this means. Aasenden and Peebles (1974), examining children 7-12 years old who had ingested fluoride supplements from shortly after birth (0.5mg/day) to the age of 3 years and 1mg/day thereafter, found that caries was virtually controlled. A follow -up study five years later (Aasenden and Peebles, 1978), on children aged 12-17 years, showed that the fluoride supplement group had a markedly lower caries experience compared with the control group. The mean caries score of the fluoride group was 80% lower than the control.

It is difficult to quantify the pre-eruptive systemic effect of fluoride supplements in the form of tablets or drops, but the topical effect from sucking a fluoride tablet for as long as possible before swallowing is likely to confer as much, if not more benefit than that attributed to the systemic effect following gastric absorption. In order to prevent the cumulative effect of fluoride intake and the risk of overdosage and fluorosis, prescribed dosage must take account of local fluoride availability, particularly in the water supply.

As far as dietary supplements are concerned, patient compliance may be a problem. Hargreaves *et al.* (1988) found that less than 50% of children on a fluoride supplement program adhered faithfully to their fluoride drop regime.

(II) FLUORIDATED SALT

Extensive studies by Toth in the early 1970's, using 250ppm fluoride in table salt, showed substantial caries-preventive effects (Toth, 1971 and 1976). The individual variation in salt intake, however, is much greater than in drinking water. In the early years when fluoride is needed most, salt intake is at its lowest level. The attention, in recent years, to the role of salt in hypertension and cardiac disease may account for some of the reasons why salt fluoridation has not been actively pursued.

(III) FLUORIDATED MILK

Milk fluoridated to contain 1.5mg of fluoride per 200ml of milk and given on 200 school days per year, has a topical benefit similar to that of water fluoridation (Stephen *et al.*, 1981). Milk, however, is not accepted universally by individuals and lactose intolerance is a particular problem amongst black communities in developing countries.

(IV) FLUORIDATED TOOTH PASTE

There is strong evidence to support the role of fluoridated dentifrices in the improvement of dental public health in the Western countries during the last two decades (Brunnelle and Carlos, 1982; O'Mullane, 1982). 1500ppm is the maximun recommended level of fluoride in dentifrices in Europe, but there is a dose response effect with only small increases in effectiveness at concentrations higher than 1000ppm. A range of preparations has been used including sodium fluoride, stannous fluoride and sodium mono-fluorophosphate. Almost all of these have yielded considerable reductions in caries experience but high concentration dentifrices (1000 ppm and above) should not be used unsupervised by children under five years of age as there is a risk of dental fluorosis from their ingestion.

(V) FLUORIDE MOUTHRINSES

The use of fluoride mouthrinses has recently become one of the most widely employed caries preventive public health methods. During the past ten years, the prevailing opinion has been that frequent topical fluoride application at low concentrations is preferable to less frequent fluoride application at higher concentrations.

The current regimes are 0.05% sodium fluoride (230ppm) daily *(a low potency high frequency application)* or 0.2% sodium fluoride (900ppm) weekly or fortnightly *(a high potency low frequency application).*

The difference between these two approaches in terms of caries prevention has been found to be insignificant (Driscoll *et al.*, 1983). It is recommended that 900ppm rinses should only be available when prescribed by a dentist, and fluoride mouth rinsing is not recommended for children under 6 years of age.

(VI) PROFESSIONALLY APPLIED GELS AND VARNISHES

Professionally applied gels and varnishes are indicated for individuals with a moderate to high level of caries activity. The fluoride concentrations of such applications vary from 970ppm (0.04% SnF_2) to 19,400ppm (8.00% SnF_2).

1.4.7 SUMMARY

Mass preventive methods, such as fluoridation of drinking water, have already proved highly effective in caries prevention. The additional availability of other sources of fluoride has increased the potential risk of chronic fluoride overdosage but the risk of pathology due to chronically high fluoride intake, e.g. crippling fluorosis or renal impairment, is very low and need not be a matter of concern (Heifetz and Horowitz, 1986). However, there is a risk of dental fluorosis with high fluoride intake.

1.5 DENTAL CARIES AND ORTHODONTIC TREATMENT

1.5.1 INTRODUCTION

During fixed appliance orthodontic treatment, dental plaque may accumulate around brackets and bands (Zachrisson, 1974), hindering passive and active oral hygiene measures, thereby increasing the risk of caries at these sites. Although different preventive methods have been tried, decalcification of teeth under and around fixed orthodontic appliances has been a problem that remains unresolved .

1.5.2 CARIES LESIONS RELATED TO ORTHODONTIC TREATMENT

An orthodontic appliance may promote dental caries due to the increased number of plaque retention sites introduced into the mouth. The period of orthodontic treatment, however, often coincides chronologically with the period of general increase in dental caries during early adolescence (Bach, 1953,1954). Caries experience, however, need not be appreciably greater in orthodontic patients than in the general population, even though the distribution of the lesions may be different.

There are 26 *in vivo* studies in the literature since the 1970's dealing with the prevalence, distribution and severity of caries lesions related to orthodontic treatment. These are summarised in Table 1.1.

From these 26 *in vivo* studies, the following conclusions can be drawn :

1. *prevalence of decalcification*

The prevalence of decalcification in patients who have completed orthodontic treatment has been shown to be similar to that found in an untreated reference

TABLE 1.1 PREVIOUS STUDIES INTO DECALCIFICATION RELATED TO FIXED ORTHODONTIC TREATMENT

STUDY	STUDY DESIGN	RESULTS AND COMMENTS
Meyers (1952)	*longitudinal *direct visual assessment (dried) *copalite coating prior bonding * 548 teeth	1. 5.9% test teeth and 27.4% control showed white spot lesions. 2. upper lateral incisors most commomly affected. lower central and lateral incisors seemed to be immune.
Zachrisson and Zachrisson (1971)	*longitudinal *direct visual assessment *Caries index (von der Fehr, 1961) *fluoride rinse	1. 89% patients were affected 2. On an overall basis, the number of new enamel alternations was small, and the total caries frequency was not markedly influenced by the appliances. 3. The vast majority of the carious lesions were observed in comparatively few patients. 4. The distribution of the lesions was significantly different in the test and control groups, both with regard to the individual tooth and the individual tooth surface. 5. Partly covered vestibular and lingual surfaces of the anterior teeth were subject to an increased caries susceptibility in comparision with the reference subjects.
Stratemann and Shannon (1974)	*direct visual assessment and indirect visual assessment (with photos) *decalcification -scored according to extention and severity *fluoride rinse * 110 control and 99 test patients	1. 58% white spot lesions (w.s.l) in control 2% w.s.l. in test (fully complied-daily rinsing--- 51 patients) 27% in test (overall including those who rinsed only infrequently) 2. degree of protection is a direct function of the degree of cooperation with fluoride rinse.
Zachrisson (1977)	*longitudinal *direct visual assessment -new white spots noted *O.H. instruction *fluoride rinse *sealant *composite (Concise) *47 patients (705 bonded brackets)	1. Out of the 47 patients , 39 had no lesions and 7 had new white spot lesions. 2. Only 15% children affected by new lesions, which was far better than the situation in full-banded patients. 3. Precoating etched enamel with sealant, in combination with daily fluoride mouth rinses and good oral hygiene, virtually eliminated the caries problem 4. No signs of enamel damage or discoloration for periods of up to 12 months subsequent to bracket removal.

TABLE 1.1 cont. PREVIOUS STUDIES INTO DECALCIFICATION RELATED TO FIXED ORTHODONTIC TREATMENT

Magness et al., (1979)	***longitudinal** ***direct visual assessment** ***professional application of F- every 3.15 week** ***22 patients (a total of 550teeth)**	**1. 18.2% patients , 1.3% teeth affected.** **2. Recommened that all patients should be offered the protection provided by daily use of the 0.4% SnF gel. Plus APF treatment in the surgery**
Gorelick et al., (1982)	***cross-sectional** ***direct visual assessment- Presence (and severity) or absence of white spots** ***No fluoride preventive programme** ***No oral hygiene instructions** ***121 patients**	**1.Frequency:** **Of the 121 patients studied, 49.6% had white spot lesions after treatment. 24% had white spot lesions in the reference group** **2. Distribution:** **Maxillary lateral incisors and canines , mandibular premolars were most frequently affected.** **Maxillary lateral incisors had three times as many white spots as maxillary central incisors , whether bonded or banded.** **Access to the flow of saliva and the distance from bracket to free gingival margin are factors suggested by the data.** **3. Teeth banded or bonded for a relatively short treatment interval (12 - 16 months) showed the same incidence of white spots as those involved in longer treatment.**
Mizrahi (1982)	***cross-sectional** ***direct visual assessment -photo used in sub -group** ***Scored white opacities (Curzon & Spector,1975)** ***Oral hygiene instructions** *** No fluoride supplement** ***527 patients**	**1.Frequency: Of the 527 patients examined before and 269 patients after multibanded treatment, there was a significant increase in prevalence(before 72.3%; after 84%) However, the prevalence of enamel opacities in patients who had completed treatment was similar to the control group considered to represent the general polulation.** **2. Severity: A significant increase in severity (Opacity Index before,0.125 ; after,0.200)** **3. Sex: No difference in prevalence both before and after treatment.However, male patients experienced a significantly higher increase in severity.This was associated with lower oral hygiene standards in males as compared to female patients.**

TABLE 1.1 cont. PREVIOUS STUDIES INTO DECALCIFICATION RELATED TO FIXED ORTHODONTIC TREATMENT

Glatz and Featherstone (1985)	***longitudinal *micro-hardness test *plaque scored *concise *49 teeth**	**1. Measureable demineralization, up to 75μm deep and with 25% mineral loss, was readily produced adjacent to bands or brackets in vivo in 4 weeks. 2. Mineral loss more pronounced near gingival margin 3. There was no relationship between mineral loss and oral hygiene index or the initial fluoride content of enamel.**
Artun and Thylstrup (1986)	***longitudinal *indirect visual assessment *SEM study**	**1. After debonding, lesions changed from chalky-white to a more diffuse opacity particularly in the peripheral parts of the lesion. 2. SEM revealed a general tendency toward leveling of the surface of the lesion indicating a loss of porous tissue. It showed signs of wear. 3. This study confirmed that removal of enviromental factors which favor plaque accumulation, in addition to continuous plaque control, results in the arrest of further enamel demineralization. 4. Gradual regression of the lesion at the clinical level was primarily a result of surface abrasion with some redeposition of minerals.**
Artun and Brobakken (1986)	***cross-sectional *direct visual assessment *Caries index and Gorelick's index *Fluoride rinse *Two test groups(60 in each group), and a referene group of 60 patients**	**1. 45% non-orthodontic controls were affected. 60% 1 year post-orthodontics 40% 1.8years post-orthodontics 2. Distribution: Majority of the lesions were scored in gingival areas. Especially affected teeth were maxillary lateral incisors and mandibular canines and premolars**
O'Reilly and Featherstone (1987)	***longitudinal *direct visual assessment, no index used *Oral hygiene instructions * 4 study groups (C)1,000ppm F- tooth paste only (1) + daily F- rinse 0.05% NaF (2)+ weekly APF treatment (3) +daily NaF and weekly APF *mineral profile- microhardness *Concise (3M) *20 patients (58 premolars)**	**1. Demineralization occuring around orthodontic appliances after only one month, even with the use of 1, 000 ppm NaF tooth paste (C) 14% mineral loss(control) (1) normal (2) normal (3) normal (last two groups showed hypermineralization) 2. Visual examination did not reveal any caries, mottling or white spots both prior to bonding or before extractions - suggests that considerable mineral loss can occur without being observed by the clinician. 3. The combination of daily brushing with a fluoridated tooth paste, coupled with daily rinsing with a fluoride(0.05% NaF) mouthrinse,will provide complete protection for the orthodontic patient by inhiting demineralization or by promoting remineralization, on surfaces at risk**

TABLE 1.1 cont. PREVIOUS STUDIES INTO DECALCIFICATION RELATED TO FIXED ORTHODONTIC TREATMENT

Geiger et al., (1988)	***cross-sectional** ***direct visual assessment** ***Fluoride rinse** ***Oral hygiene instructions** ***101 cases (1,567 teeth)**	**1.Frequency:** **Of the 101 cases,34(33.8%) had one or more teeth with white spot formation.** **Of the 1,567 teeth examined, 117(7.5%) had some white spots.** **The frequency of occurrence of white spots indicated a broad distribution (i.e. no undue influence of susceptibility in certain patients).** **Neither age nor sex showed any correlation with the incidence of white spot lesion.** **2. Distribution: Maxillary anterior segments and the mandibular posterior segments were the most frequently affected. Maxillary lateral incisors and canines were most frequently affected (17.5% and 12.2% respectively). Mandibular first premolar was affected 13.6% and the mandibular first molar 13.6%.** **3. Severity: Maxillary lateral incisors were the teeth most susceptible to more severe white spot formation. There appears to be a fairly consistent pattern of more severe white spots on the left side of the dentition as compared with the right.** **4. Duration of treatment: The incidence and severity of white spot formation were related to the length of time teeth are bracketed. All moderate and severe white spots occured when brackets were bonded for more than 24 months.** **5.Compliance: Despite efforts to educate patients and parents, poor compliance with a preventive fluoride rinse programme occured in 50% of patients. There was a significant association between poor compliance and the formation of white spots.** **6. Effect of a fluoride programme: Decalcification of the labial(buccal)surfaces of teeth during orthodontic treatment can be significantly reduced by the consistent use of a 0.05% sodium fluoride rinse during treatment.** **The one-time topical application of acidulated phosphate fluoride gel immediately after bonding appears to be of little benefit in reducing the incidence of white spots.**

TABLE 1.1 cont. PREVIOUS STUDIES INTO DECALCIFICATION RELATED TO FIXED ORTHODONTIC TREATMENT

Ogaard (1989)	***cross-sectional *direct visual assessment *Gorelick's index ,1982 *fluoride rinse * 51 orthodontic patients and 47 untreated subjects(control)**	**1. Decalcification : 95% of patients 5-7 years post-treatment; 85% non-orthodontic controls. 2. 5.7years had elapsed after completion of treatment. The median white spot score was significantly higher in the orthodontic group than in the untreated group. 3. Orthodontically treated subjects had more teeth with white spot lesions than the untreated group 4. The highest prevalence was noted on the first molars in both groups. 5. Mandibular canines and premolars and maxillary lateral incisors were also affected in the orthodontic group. 6. White spot lesions after orthodontic treatment with fixed appliances may present an esthetic problem , even more than 5 years after treatment.**
Sonis and Snell (1989)	***longitudinal *direct visual assessment (scored at end of treatment) * index after Mizrahi,1982 *22 patients (206 test and 206 control teeth)**	**1. Decalcification: 12.6% Aurafil 0% experimental F- adhesive (FluorEver OBA) 2. Fluoride ions in the test material are released by a diffusion or dissolution mechanism. 3. A decrease in the amount of decalcification around orthodontic brackets bonded with FluorEver OBA. 4. Bracket retention rates of the test material were similar to the control group over a 2-year period.**

TABLE 1.1 cont. PREVIOUS STUDIES INTO DECALCIFICATION RELATED TO FIXED ORTHODONTIC TREATMENT

Underwood et al., (1989)	***longitudinal *polarized light microscopy- water and quinoline as imbibition medium *Fluoride exchanging resin(FER) * 10 patients (165 teeth bonded)**	**1.Decalcification: 2.78%of teeth bonded with FER, 1.73% bonded with Concise. 2.Fluoride ions in the test material are released by exchanging with other anions in the oral enviroment. 3.Using FER, there was a 93% reduction in the first stages of enamel alternation(dark zone formation was 33.5% for Concise and 2.78% for the FER). 4.There was a significant reduction in the progression of early lesions with the use of FER(translucent zone progression to dark zone was found in only 6.8% of the FER group as compared with 65.2% of the control group). 5.The primary cause of failure for both adhesives was occlusal interference 6.All bond failures were adhesive rather than cohesive indicating that structural integrity was maintained for both adhesives. 7.Control and test materials have similar durability characteristics.**
Rezk-Lega et al., (1991)	***longitudinal * direct visual assessment *O.H.I. *non-fluoride tooth-paste *motivation *micro-radiography *GIC (test) - Ketac-Cem (K-C) - Aqua- Cem (A- C) *non-fluoride cement(control)-from another study *5 patients**	**1. Lesion depth (ld) was reduced by 63% for K-C, 55% for A-C 2. Total mineral loss(^Z) was reduced by 49% K-C, 27% A-C 3. No significant differences between the two GICs with respect to lesion depth or total mineral loss value. 4. Fluoride release from GIC contributes substantially to demineralization 'reduction'. However, these cements do not provide complete caries protection in sites where access is difficult.**
Mitchell (1992)	***longitudinal * Indirect visual assessment *Caries Index (after Gorelick et al) * 24 patients, 124 teeth**	**1.Decalcification: 18% Direct (test) 19% Right-On (control) 2. Average percentage of labial tooth surface affected was 1.6%. 3. No significant difference in the prevalence of decalcification between test material , a fluoride releasing composite, and control material. 4. Direct performed as well as Right-On in terms of bracket retention.**

TABLE 1.1 cont. PREVIOUS STUDIES INTO DECALCIFICATION RELATED TO FIXED ORTHODONTIC TREATMENT

Geiger et al., (1992)	***longitudinal * direct visual assessment & indirect visual assessment * Caries Index * O.H.I. * F- rinse *206 subjects**	**1. Among the 206 subjects, 69(33.5%) exhibited one or more teeth with white spot lesions. Of the bonded teeth 11.9% showed white spot formation. 2. Greatest number of white spots occured in patients treated for more than 24 months. 3. There was a bilateral symmetry of white spot occurrence in both upper and lower arches. The relative susceptibility of individual tooth was the same as in a previous study(lateral incisors and canines in the upper; canines and first premolars in the lower being most susceptible to decalcification) 4. Poor oral hygiene was a contributor to the decalcification process. 5. Those patients who complied with the rinse programme but demonstrated poor oral hygiene did manifest a significant reduction in the incidence of white spots. 6. The use of 0.05% sodium fluoride rinse during orthodontic treatment resulted in a statistically significantly reduction of enamel white spot lesions. 7. The more closely patients adhered to the daily rinse, the more likely they exhibited a decrease in the occurrence of white spot lesions regarless of oral hygiene status 8. Only 13% patients complied fully with its use.**
Ogaard et al., (1992)	***longitudinal *fluoride tooth paste *quantitative micro-radiography *VP 862 composite with fluoride *Heliosit (non F-) *6 patients**	**1.The fluoride adhesive reduced lesion depth by about 48% more than the non fluoride adhesive. 2. Regular use of fluoride tooth pastes is insufficient to inhibit lesion development around orthodontic brackets. 3. Fluoride release was found to be pH dependent.**
Turner (1993)	***longitudinal * Index (Gorelick et al., 1982) *42 patients**	**1. Decalcification: 18.9% bonded resin with NaF(test) 25% bonded Concise (control) 2. A reduction in the incidence of white spot lesions was noted with fluoride containing composite compared to Concise, but the difference in incidence was not statistically significant. 3.The new material performed well compared with the control, showing 6.9% failure rate compared with 12.18% failure rate for Concise, although the difference was not statistically significant. 4.No difference in the amount of plaque accumulated around the bracket or in the gingival health of teeth included in the study.**

TABLE 1.1 cont. PREVIOUS STUDIES INTO DECALCIFICATION RELATED TO FIXED ORTHODONTIC TREATMENT

Marcusson et al., (1993)	***longitudinal *colour slides(indirect visual scoring) * no fluoride supplement *45 patients**	**1. Decalcification: 4.7% of all patients pre-bonding 20% in GIC (Aqua-Cem) 39.2% in composite(Unite) 2. Using GIC for bracket bonding caused a significant reduction in the number of white spots at debonding. 3. However, such a benefit was no longer evident one year after debonding.**
Millett et al., (1993)	***longitudinal * indirect visual assessment * modified DDE index * GIC(Ketac-Cem) Composite (Right-On) * 23 patients**	**1. 2.8+/- 0.3 GIC 2.7+/-0.1 Right-On 2. Only assessed teeth in upper labial segments pre- and post-theatment 3. There was a significant increase in the mean number of teeth affected by decalcification post treatment. 4. The mean extent of decalcification per tooth post-treatment increased also. 5. There was no significant difference between the GIC and composite in relation to the no. of teeth affected and the extent of decalcification 6. Bonding with GIC does not appear to reduce decalcification in vivo.**
Millett et al., (1994)	***longitudinal * indirect visual assessment * modified DDE index * GIC(Ketac-Cem) Composite (Right-On) * 19 patients**	**1.Decalcification: 2.5+/-0.8 GIC 2.3+/-0.8 Right-On 2. Assessed from debond to review. 3. There was a significant decrease in mean number of teeth affected by decalcification . 4. The mean extent of decalcification per tooth decreased also. 5. There was no difference at review between either material in relation to mean number of teeth affected or the extent of decalcification. 6. Decalcification reduces post -treatment but does not appear to be significantly affected by bonding with glass ionomer.**

TABLE 1.1 cont. PREVIOUS STUDIES INTO DECALCIFICATION RELATED TO FIXED ORTHODONTIC TREATMENT

Banks and Richmond (1994)	***longitudinal *direct visual assessment *modified index *oral hygiene instructions *no fluoride supplement *pre-trial eliminating any white spot * 40 patients in each group(viscous sealant and non-viscous sealant) 289 test teeth and 282 control teeth in the viscous sealant group 306 test teeth and 305 control teeth in the non-viscous sealant group**	**1. 75% in total affected by demineralization. viscous sealant reduced the extent of decalcification by 13%(Maximum Cure) non-viscous sealant did not produce any significant difference (Transbond resin) 2. Over 60% decalcification occured in the gingival area 3. Maxillary lateral incisors and canines, and mandibular canines and second premolars were most severely affected and most likely to benefit from sealing.**
Marcusson et al., (1995)	***longitudinal *indirect visual assessment *modified index of Geiger et al.(1988) *no fluoride supplement *white spots recorded pre-bonding(n=222), at debonding(n=222) 1 year post bonding(n=214) , 2 years post bonding(n=160) n=number of teeth *60 patients (upper laterals and lower canines were assessed). *treatment time=8-39 months *mean age=13 years 7 months *Test material= Glass ionomer(Aqua-Cem) Control material= diacrylate(Unite)**	**1. At debonding white spots were found in 24% of surfaces bonded with the cement, significantly lower than 40.5% bonded with the control material. 2. At 12 months recall after debonding, the frequency of surfaces with white spots was 22.4% (GIC) and 24.2%(control). Significantly highly than before treatment. 3. At 24 months recall after debonding, the frequency of surfaces with white spots was 16.2%(GIC) and 28.7%(control).Significantly higher than before treatment. 4. Neither sex nor treatment time affected the results of group differences.**
Silverman et al., (1995)	***longitudinal *<u>no mention of</u>: method of assessment hygiene measure fluoride supplement *treatment time= 8 months *152 patients (3226 teeth) *compomer (Fuji ortho LC)**	**1. No decalcification was observed on any teeth on debonding. 2. Fluoride release protects teeth against decalcification. 3. Bonding with Fuji ortho LC saves clinical time, more patient comfort, easy to use and repair.**

group considered to represent the general population (Mizrahi, 1982). Other studies have found that between 34% and 50% of patients experience decalcification during fixed appliance orthodontic treatment. Gorelick *et al.* (1982) found 49.6% of his 121 patients developed white spot lesions when a preventive fluoride programme and oral hygiene instruction were withheld during treatment. When both a fluoride mouth rinse and oral hygiene instruction were prescribed during treatment, Geiger and co-workers (1988) recorded that 33.8% of their 101 patients suffered from demineralisation.

2. *Distribution of lesions*

Maxillary lateral incisors and canines as well as mandibular premolars have been most frequently affected by decalcification (Meyers, 1952; Gorelick *et al.*, 1982; Geiger *et al.*, 1988; Ogaard, 1989; Banks and Richmond, 1994).

Maxillary lateral incisors have been shown to have three times as many white spots as maxillary central incisors, whether bonded or banded (Gorelick *et al.*, 1982). Lower central and lateral incisors seem to be immune (Meyers,1952).

Severe white spots have been found on the left side of the dentition compared to the right hand side (Geiger *et al.*, 1988). The majority of the lesions have been found in gingival areas (Artun and Brobakken, 1986; Banks and Richmond, 1994) while the extent of mineral loss (Glatz and Featherstone, 1985) has been found to be more pronounced in this region.

3. *Treatment time*

Over an observation time of one month O'Reilly and Featherstone (1987) noticed that demineralisation occurred around orthodontic appliances even when patients used 1,000ppm NaF toothpaste. Micro-hardness assessment has shown that lesions of up to 75*u*m in depth with 25% mineral loss may be

produced readily adjacent to bands and brackets *in vivo* in 4 weeks (Glatz and Featherstone, 1985).

Gorelick *et al.* (1982) found that teeth banded or bonded for 12-16 months showed the same incidence of white spots as those involved in longer treatment. This conflicts with the later findings of Geiger *et al.* (1988) who stated that the incidence and severity of white spot formation were related to the length of time teeth were bracketed. All moderate and severe white spots occurred when brackets were bonded for more than 24 months.

4. *Bonded vs banded appliance*

Zachrisson (1977) comparing the results of his two studies in 1971 and 1977, concluded that there was less decalcification with a bonded appliance than with a banded appliance as only 15% of children were affected by new lesions with a bonded appliance compared to 89% with banded appliances.

5. *Age*

Geiger *et al.* (1988), in their study which involved 101 subjects (with 24% up to age 11years, 60% up to the age of 14 years and 16% older than 14 years), observed that age had no correlation with the incidence of white spot lesions.

6. *With fluoride releasing composite cement*

A decrease in the amount of decalcification around orthodontic brackets bonded with fluoride releasing composites was found in three *in vivo* studies (Sonis and Snell, 1989; Underwood *et al.*, 1989; Ogaard *et al.*, 1992).

Conflicting results were reported, however, by Mitchell (1991) and Turner (1993) who found that the difference in incidence of white spots between the test (fluoride-releasing cement) and control (nonfluoride-releasing cement) groups was not statistically significant.

7. *With fluoride releasing Glass ionomer cement*

The glass ionomer cement, Ketac-Cem, has been found to reduce demineralisation significantly four weeks post bonding, both in terms of lesion depth and total mineral loss (Rezk-Lega *et al.*, 1991). A significant reduction in white spot lesions (Marcusson *et al.*, 1993) was observed also after 8-29 months orthodontic treatment using Aqua Cem as a bonding agent. However, in another *in vivo* study, Millett *et al.* (1993) did not record a reduction in decalcification after 14.5 +/- 5.2 months of treatment when the GIC, Ketac Cem, was used for bracket bonding. Despite the fact that Ketac Cem released more fluoride than Aqua Cem *in vitro* (Creanor *et al.*, 1994), this was not reflected in the *in vivo* situation when the conflicting results of Millett *et al.* (1993) and Marcusson *et al.* (1933) are compared. This may be due to the difference in sample size between the two studies (45 subjects in Marcusson *et al.*'s study versus 23 in Millett *et al.*'s study).

8. *Regression of white spot lesions*

Regression of white spot lesions after orthodontic treatment occurs to different degrees and it may take a long period of time for complete regression to occur. White spot lesions may still present an aesthetic problem even more than 5 years after treatment (Ogaard, 1989).

Millett *et al.* (1994) have shown that there was a significant decrease in the mean number of teeth affected by decalcification at one year review after debonding. This gradual regression of the lesions may be primarily a result of surface abrasion with some redeposition of minerals (Artun and Thylstrup, 1986).

1.5.3 EFFECTIVENESS OF DIFFERENT CARIES PREVENTION MEASURES IN ORTHODONTICS

Much interest has been channelled into ascertaining an effective method to prevent or reduce the incidence of demineralisation during orthodontic treatment. The common approaches have included oral hygiene measures, fluoride supplements, fluoride varnish application to the enamel surface and fluoride releasing cements. These will be considered in turn.

1. *oral hygiene measures*

Although no strong connection between oral cleanliness and dental caries in the general population has ever been demonstrated (Koch *et al.*, 1986), poor oral hygiene has been shown to contribute to decalcification during orthodontic treatment (Geiger *et al.*, 1992; Zachrisson, 1977; Mizrahi, 1982).

Weekly professional removal of dental plaque has been shown to suppress bacterial activity and hence caries development (Holmen *et al.*, 1988). On the other hand, in the same study, results indicated that after 5 weeks with completely undisturbed plaque accumulation there was visible enamel demineralisation in all subjects.

In a cross-sectional study, Mizrahi (1983) observed that male patients suffered a significantly higher increase in *severity* of decalcification following fixed appliance treatment. He concluded that this was associated with lower oral hygiene standards in males compared to female patients. Gorelick and co-workers (1982) in their study of "white spot" formation after orthodontic treatment also stated that inadequate oral hygiene on labial surfaces was directly related to undesirable decalcification. They found that despite efforts to educate patients and parents, poor compliance with oral hygiene measures was shown to be associated with a higher prevalence of decalcification.

2. *fluoride supplements*

Most studies have found that fluoride supplements reduce significantly the incidence of decalcification (Zachrisson and Zachrisson,1971; Stratemann and Shannon, 1974; Zahrisson, 1977; Geiger *et al.*,1988). Different preparations (e.g. APF, NaF, SnF_3), concentrations, frequency of administration and mode of application have been examined .

Stratemann and Shannon (1974) found that the degree of protection gained by fluoride supplementation during treatment was a direct function of the degree of co-operation. The prevalence of white spot lesions was only 2%, in those who used the fluoride mouth rinse daily. This increased to 27% for those who only used it occasionally and 58% for those who do not use it at all.

One particularly comprehensive study conducted by O'Reilly and Featherstone (1987) assessed the effectiveness of four different fluoride regimes and concluded that the combination of daily brushing with a fluoridated tooth paste, coupled with daily rinsing with a fluoride (0.05% NaF) mouthrinse, provided complete protection against decalcification. This is consistent with the findings of Geiger *et al.* (1988) which stated that decalcification of the labial (buccal) surfaces of teeth during orthodontic treatment can be reduced significantly by the consistent use of 0.05% sodium fluoride rinse during treatment. These workers found, however, that only 50% of patients adhered faithfully with the rinsing programme despite efforts to motivate patients and parents alike.

The one-time topical application of acidulated phosphate fluoride gel immediately after bonding appears to be of little benefit in reducing the incidence of white spots (Geiger *et al.*, 1988). It seems that for orthodontic patients undergoing fixed appliance therapy, 'high frequency low

concentration' fluoride treatment works better than a 'low frequency high concentration'.

3. *application of topical fluoride to etched enamel*

Recent evidence indicates that one of the major mechanisms by which fluoride reduces caries incidence is remineralisation. Remineralisation reduces the size of the lesion and makes it more resistant to acid dissolution of apatite crystals.

Many workers (Kochavi *et al.*, 1975; Thornton *et al.*, 1986 and Takahasi *et al.*, 1980) have advocated the addition of (0.01%-2%) sodium fluoride to the etchant to promote remineralisation. Others (Low *et al.*,1975 and Bohrer *et al.*,1980) have favoured the application of topical fluoride after etching but before bonding while Lehmen *et al.*(1981) recommended that topical fluoride be applied after bonding. The main objective has been to reharden and remineralise the etched enamel, without interfering with bonding.

4. *coating enamel surface with a sealant*

Placement of a resin sealant on the enamel surface before bracket bonding has been suggested as a means of providing protection against demineralisation, particularly at known sites of plaque accumulation. The effectiveness of sealant protection depends on the clinical life of this thin coating. Zachrisson (1977) found that precoating etched enamel with a sealant, in combination with daily fluoride mouth rinses and good oral hygiene, virtually eliminated the caries problem. Nonetheless, Ceen and Gwinnett (1979) observed that thin resin films with their low abrasion resistance, cannot be expected to provide long-standing protection against demineralisation.

Recent studies on sealants have, however, shown them to be quite effective in reducing decalcification. Buyukilmaz *et al.* (1994) found in a 4-week *in vivo* study, that 1% titanium tetrafluoride sealant conferred a thin coating on the

enamel surface that reduced significantly lesion depths and total mineral loss at the bracket periphery. Similarly, Banks and Richmond (1994) found 13.1% less decalcification using a viscous sealant.

5. *fluoride releasing cements*

These have several clinical advantages, including slow-release of low levels of fluoride, site specificity, and are independent of patient compliance. Recent research with this class of cements will be reviewed in the next section.

1.5.4 SUMMARY

A high percentage of orthodontic patients experience decalcification during fixed appliance treatment. As regression of such lessions can take a considerable time and may only be partial, oral hygiene measures and fluoride rinses should be included in the preventive regime for all patients to minimise and hopefully prevent such iatrogenic damage.

1.6 HISTORY, DEVELOPMENT AND COMPOSITION OF FLUORIDE-RELEASING CEMENTS

1.6.1 INTRODUCTION

Concern amongst the orthodontic profession about decalcification during orthodontic treatment has led to the development of a number of adhesive materials claiming to release fluoride, which may help resolve this problem. Although the level of fluoride release required to achieve clinical efficacy has not been established, it have been shown *in vitro* (Forss and Seppa, 1990) that fluoride released from some materials prevents demineralisation of enamel. Besides that, a less cariogenic flora has been found in plaque deposits adjacent to a fluoride releasing cement in orthodontic patients (Hallgren *et al.*, 1992) .

1.6.2 IDEAL PROPERTIES OF ORTHODONTIC CEMENTS

Cements used in orthodontic bonding must be able to fulfil the basic clinical requirements. The desirable properties of an orthodontic luting cement are listed in Table 1.2. Although many dental cements have been developed and used in orthodontics, only those cements which have been used for bracket bonding or which have incorporated fluoride will be considered here.

1.6.3 ZINC POLYCARBOXYLATE CEMENTS

Zinc polycarboxylate cement was developed by D.C. Smith (1968). These cements, which are adhesive to both enamel and stainless steel, have been used for the cementation of orthodontic brackets.

The powder consists mainly of zinc oxide, though a small amount of magnesium oxide may be present. The liquid is an approximately 40% aqueous solution of polyacrylic acid. The setting reaction involves the formation of zinc polyacrylate. This set material is a cored structure containing a considerable quantity of unreacted zinc oxide. It is almost as strong as phosphate materials in compression , and stronger in tension. Under ideal conditions, the adhesion of a polycarboxylate to a clean dried surface of enamel is more than twice that of a conventional zinc phosphate cement (Combe, 1981). Stannous fluoride is a constituent of some of these cements, which has the potential of releasing fluoride.

1.6.4 GLASS IONOMER CEMENTS

Glass ionomer cements (GIC) were invented by Wilson and Kent (1972) as a hybrid of the silicate and polycarboxylate cements. They became available for clinical use in Europe around 1975.

Table 1.2

Desirable properties of an orthodontic luting cement

1. Appropriate working time and good handling properties.
2. Good physical properties.
   - adhesive.
   - good compressive and tensile strength.
   - a coefficient of thermal expansion appropriate for the tooth and metal.
3. Thin film thickness.
4. Low solubility in oral fluids.
5. Non-toxic and biocompatible to oral tissues.
6. Good wetting of the band/bracket surface and tooth surface.
7. Release and uptake of fluoride.

The powder in the early glass ionomer cement was calcium aluminosilicate glass with a high fluoride content. The liquid was the same as that in polycarboxylate cement: a co-polymer formed by adding itaconic acid to polyacrylic acid. Tartaric acid was added to give a sharp setting time (Crisp and Wilson, 1976). On mixing powder and liquid, calcium and aluminum ions are extracted from the surface of the powder particles. Ca^{2+} and Al^{3+} ionically cross-link the polyacrylate chains, causing the cement to gel, set and harden. The set material contains opal glass particles, sheathed in a siliceous gel, embedded in a metal polyacrylate matrix.

Many improvements have been made since GICs were first marketed as Aspa Cement. In the water-hardening (anhydrous) glass-ionomer cement, the liquid component of the polyacrylic acid is included in the powder as a polymer. Some manufacturers today use maleic or other acids in the place of itaconic acid. As far as a luting cement is concerned, the film thickness has become smaller.

The advantages and disadvantages of Glass ionomer cement (GIC) as a luting cement are shown in Table 1.3.

While conventional GICs have been used successfully to retain orthodontic bands (Durning, 1989; Stirrups, 1991), their utilisation as a bracket adhesive has been disappointing (Fricker, 1992). Fricker, (1992) reported a failure rate of around 20% during a 12 month evaluation of two GICs. He concluded that this level of bracket failure was unsatisfactory for routine clinical use, although he suggested that GIC could be considered for patients at risk of decalcification during orthodontic treatment.

As water sensitivity, solubility and bond strength are the major problems with conventional GICs, modifications of these cements by the inclusion of various

Table 1.3

Advantages and disadvantages of Glass ionomer cement (GIC) as a luting cement

Advantages:

1. Adhere to both enamel and stainless steel.
2. Good mixing consistency in the anhydrous (water-hardening) type with a rapid 'snap' set.
3. Compressive strength of GIC is about 1500-2100Kg/cm ^{2} , a higher value than obtained by zinc phosphate cement (e.g. Ketac-Cem 1200 Kg/cm^2) - i.e. related to mechanical bonding and interlocking
4. Coefficient of thermal expansion of GIC (13 x $10^{-6}/^{o}C$) resembles that of tooth structure (11 x $10^{-6}/^{o}C$).
5. Finer powder particles improved flow . Recent products have achieved the same film thickness as zinc phosphate cement.
6. Release more fluoride than silicate cements (Forsten, 1977)
7. Fluoride uptake following topical fluoride application (Creanor,1994).

Disadvantages:

1.Solubility of GIC in pure water is 0.08-0.40% which is unfavourable compared to the 0.03% of zinc phosphate cement. Very high leakage and dissolution of cement can occur if initial setting is not protected (Meyers, 1983).
2. Bond strength between enamel and GIC is considerably lower than that obtained between acid etched enamel and composite resin.

resins has led to the development of a new generation of hybrid resin-modified glass ionomer cements called 'compomers'. The development, characteristics and clinical potential of the compomer materials will be discussed later in section 1.6.6 .

1.6.5 RESIN BASED CEMENTS

The development of the acid etch technique (Buonocore, 1955) led to the introduction of direct bonding of orthodontic brackets with composite resins. Resin based cements were used to cement bands and brackets in the 1970's. These early materials were either acrylic or diacrylate resins. Their high coefficient of thermal expansion (approximately 10 times that of tooth substance) and high setting contraction (6-7%) are their main disadvantages (Newman, 1969).

Bowen (1963) developed a resin material based on Bis-GMA . This material can undergo free radical addition polymerization to give a rigid cross-linked polymer. With the use of silane coupling agents, which bond the polymer and the fillers together, mechanical properties have greatly improved (Combe,1981). The introduction of fillers of different size has reduced shrinkage on setting, reduced the coefficient of thermal expansion as well as improving hardness and compressive strength (van Noort, 1994). Viscosity of a composite luting cement depends on the filler content. Optimum composition of fillers ensures good 'wetting' of etched enamel and the bracket base while the film is thick enough to prevent the bracket from 'creeping'.

The common components of composite material are listed in Table 1.4.
Composite materials used for orthodontic bonding, like filling materials, are available in different systems. The ones currently used are :-

1. Two-paste systems, one containing an activator and the other the initiator, which are mixed together (e.g. Concise, 3M Unitek UK). Multiple mixes

Table 1.4

The common components of composite material

1. Principle monomer :	- bisphenol-A glycidyl methacrylate (BIS-GMA)
	-urethane dimethacrylate
2. Diluent monomer:	-methyl methacrylate (MMA)
(viscosity controller)	-ethylene glycol dimethacrylate (EDMA)
	- triethylene glycol dimethacrylate (TEGDMA)
3. Inorganic fillers:	lithium aluminosilicates, crystalline quartz, barium aluminoborate silica glass, barium glass, strontium glass etc. A wide variety of fillers have been used for different purposes. Particle size varies from 0.02μm (micro-fine) to 40μm.
4. Coupling agent:	Silane coupling agent
5. Initiator/activator:	(i) chemical activation: benzoyl peroxide initiator and tertiary amine activator , N,N-di hydroxy-ethyl-p-toluidine
	(ii) U.V.light activation: benzoin methyl ether
	(iii)visible light activation: α- diketone amine
6. U.V. Stabilizer:	2-hydroxy-4-methoxybenzophenone
7. Inhibitor :	hydroquinone

are required to complete a full mouth bond-up which is time-consuming. Besides that, air bubbles can become incorporated into the composite during mixing which can weaken its mechanical properties.

2. No-mix systems, with the initiator in a fluid which is painted onto the etched enamel and the bracket base. The activator is contained in a paste which is then applied to the bracket (e.g. Right-On T.P.Orthodontics). Polymerization is achieved when the bracket is pushed firmly into place. A thinner layer of cement under the bracket base can be achieved by using brackets with compound contoured bases, which in turn ensures complete polymerization.

3. Light-cured systems (e.g. Durafil) are becoming more popular as working time is easier to control. However, to ensure complete polymerization, up to 40 seconds of light activation may be required (O'Brien *et al.*, 1989) and the light source should be held as close to the bracket as possible and positioned from different directions (Wang and Meng, 1992). While this technique allows command setting, a full mouth 'bond-up' can take a considerable time and complete polymerization cannot be ensured.

4. Dual-cured systems combine the command setting characteristic of the light-cured system with the chemical setting reactions of the self-cured composite materials. They can be either polymerised entirely by visible blue light; partially polymerised by light with chemical polymerisation completing the setting reaction; or left to auto-polymerize. The chair-side time required is less as curing time with blue-light is reduced.

5. Fluoride-releasing composites (e.g. Fluorever) have been developed in response to concern about decalcification during orthodontic treatment. Fluoride is either incorporated in a dispersed phase, which is released by

dissolution (Forsten *et al.*, 1972) or fluoride is released by an ion-exchange mechanism (Rawls *et al.*, 1983).

1.6.6 RESIN-MODIFIED GLASS IONOMER CEMENTS

Resin-modified glass ionomer cements, 'compomers', are hybrid materials of glass ionomer cement and composite resin. Properties of this class of material range between those of conventional acid/base setting glass ionomers on one hand and free-radical cured composites on the other. Some of the recently marketed products include Vitremer, Dyract, Fuji II LC, Variglass, Photac-fil. This class of material is distinguished from 'fluoride-releasing resins' by the presence of an acid/base setting reaction (Wilson *et al.*, 1985).

Earlier glass ionomer cements were known to have drawbacks, such as low tensile strength, water sensitivity and solubility. Modifications of the setting reactions through the inclusion of various resins have led to the development of the resin-modified cements. The setting reaction of the resin components is light activated (e.g. Dyract) and provides some degree of protection to the acid/base setting reaction of the glass ionomer cement, greatly reducing its water sensitivity.

However, uncured resin which is too far from the light source may remain soft and introduce mechanical weaknesses in the cement. Further improvements have been made by incorporating an additional chemical setting reaction so that any remaining resin which is not affected by light activation will undergo a dark cure reaction - hence the term 'tri-cure' used in some products (e.g. Vitremer). With the inclusion of resins, the hybrid materials may become increasingly less like glass ionomers and more like composite resins, depending on the proportion of each of these materials incorporated.

Properties of resin-modified glass ionomer cements can be summarised as follows:

1. Bonding to tooth structure (e.g. enamel and dentine) and metal (e.g. stainless steel).
2. Light initiated, command setting, available in dual-cure and tri-cure forms.
3. Fluoride-releasing.
4. Significantly greater early compressive strength and tensile strength than conventional glass ionomer cements.
5. More resistant to moisture and desiccation than conventional glass ionomer cements.

1.6.7 SUMMARY

There are obvious advantages of fluoride-releasing cements in orthodontics: the active agent, fluoride, can be delivered directly to or near the vicinity of the bracket margins where plaque accumulates; the active agent can be maintained continuously at the site of need over an extended period; and reliance on patient cooperation with alternative preventive programmes can be reduced or eliminated.

To this end, manufacturers have concentrated on developing fluoride-releasing composites and resin-modified glass ionomer cements. There are now a variety of orthodontic luting cements which claim to release fluoride while maintaining a reliable bond strength. Their performance *in vivo* and *in vitro* will be examined in the next section.

1.7 REVIEW OF FLUORIDE RELEASING CEMENTS IN ORTHODONTICS

1.7.1 INTRODUCTION

The inhibitory effect of fluoride on bacterial activity and on demineralisation of enamel have been well established and were discussed in previous sections. Fluoride enriched zinc phosphate and zinc polycarboxyate cements have been used to retain orthodontic bands. Glass ionomer cements and some composite resins have been used also to retain orthodontic brackets as well as bands. The controlled release of fluoride from these cements and their site specific characteristics have been the subject of extensive *in vitro* and *in vivo* investigations. The *in vivo* studies are summarized in Table 1.5.

1.7.2 RESIN-BASED CEMENTS *IN VITRO*

Fluoride-releasing composites have a weaker bond strength and lower retention rates due to the dissociation of the material as fluoride is released (Chan *et al.*, 1990). Only a small amount of fluoride is released and the quantity of fluoride released depletes rapidly (Fox, 1990).

Bishara and Chan (1991) noted the fluoride releasing pattern of a light-activated fluoride-releasing composite (FluorEver OBA). It was found that a mean of 2.6 ppm was released on day 1, decreasing to a mean of 0.42ppm by day 2 and to 0.04 by day 43. The decline in the first 24 hours was most dramatic and it continued to decline to the end of the test period. The clinical significance of these low but constant and site-specific fluoride concentrations was unclear.

Table 1.5

Previous in vivo studies of fluoride releasing cements related to fixed orthodontic treatment

Study	Material	Sample size	Treatment period	Comments
Sonis & Snell, 1989	F- releasing resin (FluorEver OBA)	22 patients (412 teeth)	25 months	*0% decalcification with fluoride releasing composite. *12.6% decalcification with non-fluoride releasing control.
Underwood et al., 1989	F- releasing resin	10 patients (165 teeth)	60 days	*Significant reduction in the formation and progression of early lesions.
Ogaard et al ., 1992	F- releasing resin (VP 862)	6 patients (20 teeth-premolars)	4 weeks	* F- releasing resin reduced lesion depth by about 48%.
Mitchell, 1992	F-releasing resin	24 patients (124 teeth)	10.5 +/- 4.2 months	*No significant difference between test and control material.
Eliades et al., 1992	F-releasing resin (VP 862)	10 patients (16 teeth)	9 months	*No significant difference between test and control groups regarding fluoride uptake in enamel .
Turner, 1993	F-releasing resin	42 patients (406 teeth)	1.6 years	*A reduction in white spot lesions, but no significant difference between the F-releasing material and the control material.
Rezk-Lega et al., 1991	Glass ionomer cement (GIC) (Ketac-Cem[KC] Aqua-Cem[AC])	5 patients (18 premolars)	4 weeks	*Reduction in lesion depth (63%KC, 55%AC). *Reduction in total mineral loss(49%KC, 27%AC). *No significant difference between KC and AC.
Marcusson et al ., 1993	GIC Aqua-Cem [AC]	45 patients	22 months	* A significant reduction in white spot lesions.

Table 1.5 cont

Previous in vivo studies of fluoride releasing cements related to fixed orthodontic treatment

Millett et al., 1993	GIC Ketac-Cem[KC]	23 patients	14.5+/-5.2	*Bonding with GIC did not reduce decalcification
Marcusson et al., 1995	GIC Aqua-Cem	60 patients	8-39 months	*A significant reduction in white spot lesions bonding with GIC. *At 12 and 24 months recall after debonding, white spots in both GIC and control groups were significantly more than before treatment.
Silverman et al., 1995	Compomer Fuji Ortho LC	152patients	8 months	*No decalcification was observed on any teeth.

Creanor *et al.*(1994) and Ghani *et al.*(1994) suggested that fluoride-releasing composites may have a caries preventive effect around orthodontic brackets. Teeth bonded with Mirage Dual Cure (a fluoride-releasing composite) had less white spot formation than those bonded with other cements. The mineral loss of the lesions in the teeth bonded with the fluoride-releasing cement was also much less than that of the lesions in the non-fluoridated group.

1.7.3 RESIN-BASED CEMENTS *IN VIVO*

Sonis and Snell (1989) in their study, lasting 25 months, found no decalcification around orthodontic brackets bonded with a fluoride-releasing composite (FluorEverOBA) compared with 12.6% of decalcification in the control group bonded with a conventional composite (Aurafil).

Underwood (1989) studied a fluoride-exchanging resin (FER) and concluded that by using FER, there was a 93% reduction in the first stages of enamel alteration 60 days post bonding and also a significant reduction in the progression of early caries lesions.

Ogaard *et al.* (1992) confirmed, by using a microradiography technique, that a fluoride bonding adhesive reduced lesion depth by about 48% more than the non fluoride adhesive in a 4 week trial period.

On the contrary, the experimental fluoride-releasing orthodontic adhesive VP-862 showed a very low rate of fluoride incorporation in human enamel (Eliades *et al.*, 1992). The cumulative fluoride uptake by the enamel 9 months post-bonding was not significantly different from that with the non-fluoride releasing composite.

Mitchell (1992) and Turner (1993) in their longitudinal studies (mean length of treatment time was 10.5 +/– 4.2 months and 12 months respectively) found no significant difference in the prevalence of decalcification between a fluoride-releasing composite and a conventional material.

The results from different clinical studies have been conflicting. The incomplete protection of enamel by a fluoride-releasing composite may be due to:

1. The amount of fluoride released from beneath brackets is relatively small because only the edges of a thin film of adhesive are exposed to the medium (Chan *et al.*, 1990).
2. A significant amount of fluoride is only released in the first 24 hours. The decline in the rate of fluoride release is rapid and continuous.
3. There is no uptake of fluoride from topical agents (e.g. fluoridated tooth pastes and fluoride mouth rinses).

1.7.4 GLASS-IONOMER CEMENTS *IN VITRO*

Glass ionomer cements release fluoride into the surrounding tissues, a property that have been evaluated both *in vitro* (Swartz *et al.*, 1984; Wilson *et al.*, 1985; Forsten 1990) and *in vivo* (Hattab *et al.*, 1989).

When used as a thin film, such as a luting agent, glass ionomer cements (GIC) have been found also to release a significant amount of fluoride (Muzynski *et al.*, 1988). The effect of fluoride release has been a reduction in caries (Hicks *et al.*, 1986; Serra and Cury, 1992; Gasparini *et al.*, 1991) and the reduction of Streptococcus mutans adjacent to GIC (Forss *et al.*, 1990; Benelli *et al.*, 1993).

However, not all commercially available GICs are the same; they vary in the amounts of fluoride released which effects their clinical preformance. Creanor *et al.* (1994) investigated the fluoride-releasing characteristics of three conventional glass ionomer cements (Ketac fil, Chemfil Superior and Aquacem) and two resin-modified glass ionomer cements (Fuji II LC and Vitrebond). All materials released measurable amounts of fluoride (15.3-155.2ppm) in the first day, fell sharply on the second day (range 6.3-44.3ppm) and came to a stable level (range 0.9ppm-3.99ppm) by 60 days but continued to release fluoride. Creanor's findings were in agreement with other studies (Crisp *et al.*, 1980; El Mallakh and Sarkar, 1990) regarding the pattern of fluoride release from GIC.

Creanor *et al.* (1994) and Forsten (1990) demonstrated also that GICs were able to take up fluoride and subsequently release it. The ability to take up extrinsic fluoride allows replenishment of the fluoride within the material and may, therefore, enhance the anticariogenic activity of the GIC.

Despite the ability to release and take up fluoride, the inferior bond strength of conventional glass ionomers has been confirmed in many reports (Cook and Youngson, 1988; Kimmins, 1992; Compton *et al.*, 1992) and this precludes their routine use for orthodontic bonding. Some resin-modified glass ionomers appear, however, to have an adequate and reliable bond strength for clinical orthodontic practice (Fricker, 1994; Silverman *et al.*, 1995).

1.7.5 GLASS-IONOMER CEMENTS *IN VIVO*

Rezk-Lega *et al.* (1991) in a 4-week clinical trial, found that fluoride release from the GICs (Ketac-Cem and Aqua-Cem) contributed substantially to demineralisation 'reduction'. Both lesion depth and total mineral loss were reduced significantly. However, these cements still do not provide complete

caries protection in sites where access is difficult. Marcusson *et al.* (1993) found a significant reduction also in the number of white spots at debonding (mean treatment period was 22 months) using a GIC (Aqua-Cem) for bracket bonding.

However, Millett *et al.* (1993) found that there was no significant difference between GIC (Ketac-Cem) and composite in relation to the number of teeth affected and the extent of decalcification (mean treatment time 14.5+/– 5.2 months).

1.7.6 RESIN-MODIFIED CEMENTS *IN VITRO*

Resin-modified glass ionomers, 'compomers', were developed to combine the desirable characteristics of a glass ionomer and composite resin. Lee and Kim (1995) compared the fluoride-releasing properties of four different orthodontic adhesives (Orthodontic Adhesive Bonding System, Ketac-Cem, Chemfil Superior and Vitremer) and found that all fluoride-releasing agents had a high initial release rate which then decreased exponentially followed by a further slower decline. A similar pattern of release was observed by Creanor *et al.* (1994) who tested five fluoride-releasing materials (Ketac Fil, Chemfil Superior, Fuji II LC, Aquacem and Vitrebond) and Yip (1995) who investigated other fluoride-releasing cements, namely: Dyract, Fuji II LC, Photac-Fil, VariGlass and Vitremer.

In Creanor's (1994) study, the resin-modified glass ionomer cement (Vitrebond) gave the highest level of fluoride release compared with conventional glass ionomer cements. Lee and Kim (1995) found a significantly greater amount of fluoride released from a resin-modified glass ionomer cement (Vitremer) (0.493ppm) than from the conventional glass

ionomer (Ketac-Cem) (0.269ppm) and the fluoride releasing resin (Orthodontic Adhesive Bonding System) (0.083ppm). It is interesting to note that the incorporation of resins into some brands of glass ionomer cement does not appear to compromise their fluoride releasing ability.

Chadwick and Gordon (1995) investigated the uptake of fluoride by enamel adjacent to two resin-modified glass ionomer cements by using an acid etch biopsy technique. They concluded that the concentration of fluoride in enamel adjacent to one of the cements, Vitrabond, increased significantly compared to the baseline record, while another material (Geristore) did not show any significant increase. It is unclear whether the level of fluoride increase in enamel adjacent to brackets bonded with Vitrabond would increase the caries resistance of the enamel.

1.7.7 RESIN-MODIFIED GLASS IONOMER CEMENTS *IN VIVO*

To date there has been only one report, Silverman *et al.* (1995), on the clinical preformance of resin-modified glass ionomers with regard to the prevention of decalcification *in vivo* during orthodontic treatment. They reported no decalcification with Fuji Ortho LC but it appears that no objective assessment of decalcification was made.

1.7.8 SUMMARY

Fluoride-releasing composites have been shown to offer a certain degree of protection against demineralisation around orthodontic brackets. However, their physical properties may be weakened due to the fluoride release mechanism. The majority of the fluoride is released over the first day after bonding, then declines rapidly but continues to be released in very small amounts.

Glass ionomers have been found to release more fluoride than fluoride-releasing resins. The release pattern is similar to that of fluoride releasing composites but with a higher initial release which is maintained at this level for longer. However, the bond strength of conventional glass ionomer cements needs to be improved before they can be used routinely for bracket bonding. Some of the recently marketed resin-modified glass ionomer cements have been shown to release more fluoride than either conventional glass ionomers or fluoride-releasing resin based cements. Their bond strength is improved compared with conventional glass ionomers but their *in vivo* preformance concerning prevention of demineralisation requires further investigation.

1.8 METHODS OF ASSESSMENT OF DEMINERALISATION

1.8.1 INTRODUCTION

There are now many different ways to assess the change in mineral content that occurs during demineralisation or remineralisation. Macroscopically, the enamel changes can be evaluated by direct or indirect visual assessment. The severity of demineralisation can be graded by the use of various indices. Some of the indices are more informative than others but some are not user-friendly due to the complexity of their interpretation.

Microscopically, mineral loss from enamel as a result of the caries process can be expressed in different ways (Ten Bosch and Angmar, 1991). These are:

A) The mineral loss from a specific point, expressed as a decrease in mineral concentration (Kg/m^3 or % vol).

B) The integral of the change in mineral content over a known distance from the surface of the tooth. (Kg/m^2 or % vol x μm).

C) The total mineral loss (Kg or g).

The principles, advantages and disadvantages of each method of assessment will be discussed briefly in the following sections.

1.8.2 DIRECT / INDIRECT VISUAL ASSESSMENT

Many studies have assessed the presence or absence of white spots by subjective observation alone. Visual assessment has been made of the enamel *directly* in over 70% of previous studies into decalcification related to fixed orthodontic treatment. *Indirect* visual assessment, either by monochrome or colour transparencies, has been employed by other workers (Stratemann and Shannon, 1974; Mizrahi, 1982; Millett *et al.*, 1993). There are several advantages of indirect visual assessment over direct visual assessment which are as follows:

1. Repeated assessments are possible at any time.
2. All records are taken and viewed under standardised conditions.
3. The extent and distribution of any enamel decalcification before and after the trial can be compared easily.
4. Intra-examiner and inter-examiner reliability can be assessed easily.

However, indirect visual examination is only reliable if a standardised photographic technique is employed (Hill and Mitchell, 1991). The surface condition of the enamel, either wet or dry, should be maintained the same on both pre- and post-treatment views when photographic records are taken.

Different indices have been used to assess demineralisation following orthodontic treatment and these are listed below:-

studies	*index*
Zachrisson and Zachrisson,1971	Caries index (von der Fehr,1961)
Artun and Brobakken,1986	Caries index and Gorelick's index.
Mizrahi,1982	(Curzon & Spector,1977)
Ogaard,1989	Gorelick's index

Sonis and Snell,1989	(Mizrahi,1982)
Mitchell, 1992	Gorelick's index
Millett *et al*, 1993,1994	modified DDE index
Banks and Richmond,1994	Enamel Decalcification Index (modification of Artun and Brobakken, 1986)

The Caries Index (Von der Fehr *et al.*, 1970) and its modified versions proposed by Gorelick (1982), appear to be the most commonly used indices for assessment of decalcification in orthodontic patients. The DDE Index is, however, more comprehensive and has been used in epidemiological studies of enamel opacities. It has been applied in a couple of orthodontic studies.

1.8.3 MICRORADIOGRAPHY/ MICRODENSITOMETRY

Microradiography

Microradiography is a technique which measures the absorption of monochromatic x-radiation by a thin tooth section. Absorption of monochromatic X-rays by a tooth section is dependent on the mineral content of that section. Demineralised enamel absorbs less X-radiation than sound enamel. The optical density, or relative greyness, of a radiographic plate is dependent on the mineral content of the enamel, and the lesion will appear as a dark area on the exposed radiographic plate.

Transverse radiography originated from Thewlis in 1940, and was developed as a quantitative method by Angmar *et al.* (1963). A cross-section of the tooth thickness in the order of 120-150μm, is exposed to the X-ray source. Since X-radiation absorption is proportional to the resultant optical density of the film, an aluminium step-wedge of known thickness is exposed simultaneously against which the tooth section is calibrated. Mineral content at a point in the

sample can be calculated by microdensitometry using Angmar's formula (Angmar *et al.*, 1963).

Longitudinal Microradiography employs a similar principle, but the sections are prepared in different thicknesses and exposure is made from the anatomical surface instead of in cross-section. This involves samples of teeth up to 0.5mm thick which are cut parallel to the anatomical surface.

The Longitudinal Microradiography technique can measure change in mineral profile of a section but is unable to estimate mineral content at different depths. Besides, this measurement can produce errors of up to 20% (ten Bosch and Angmar-Mannson, 1991).

Wavelength Independent Microradiography uses polychromatic, high energy X-radiation (greater or equal to 60Kv) for non-destructive mineral determination of the whole tooth. It can measure, with great accuracy, the amount of mineral per unit area in enamel with a thickness of 0.3mm to 6.00mm . However, like longitudinal microradiography, it is unable to determine the change in mineral content of a lesion with depth.

Microdensitometry

Microdensitometry is a technique which allows precise assessment of the greyness of a microradiographic image by measuring its optical density using a system comprising a microscope, TV camera and an image analyser. Mineral content of the area of interest can be obtained by analysing the grey level of the image. A constant and uniform light source is used to illuminate the microradiograph. Light transmitted through this radiographic image is registered by a light detector and a recorder. The amount of light transmitted is correlated with that obtained from the reference image which comprises a series of known thicknesses of aluminium foil.

The advantages of this technique are:

1. Quantitive measurement of mineral loss or gain can be preformed accurately.
2. Measurement of lesion depth can be made.
3. Mineral distribution can be measured.
4. Serial measurements are possible on single sections.
5. Measurements can be repeated.

The disadvantages of this technique are:

1. It is destructive, as preparation of thin ground sections is necessary.
2. It is difficult to produce a section with absolute uniform thickness (White *et al.*, 1992).
3. It is not possible to measure the mineral content of the outer 10 μm of enamel.
4. The image may be enlarged due to the use of unparallel X-ray beams.
5. X-ray beam homogenicity along the y-axis can be a problem (Creanor, 1987)

1.8.4 MICROHARDNESS

Microhardness is a technique which measures indentation produced by a diamond indenter (Knoop diamond or Vickers diamond) and assesses the indentation by an optical microscope. The hardness is related to the compressive strength applied (a given load for a known period of time), and measures the lesion's resistance to the force before breaking down. The result may be taken as a measure of mineral content of the underlying tissue (ten Bosch and Angmar-Mansson, 1991)

The diamond indenter can be placed over the anatomical surface of the lesion so that any indentation passes through the surface zone towards the lesion

body. Alternatively, a cross-sectional slice through a lesion can be used , placing the diamond perpendicular to the original surface at intervals across the surface zone and body of the lesion (Davidson *et al.*, 1974). This cross-sectional microhardness testing in enamel correlates well with the degree of demineralisation as measured microradiographically (Featherstone *et al.*, 1983). However, this technique is not as sensitive as measurements made by transverse microradiography.

Microhardness tests have been widely used on enamel slabs which have been exposed to the oral enviroment (Koulourides *et al.*,1974) as well as *in vitro* (Arends *et al.*, 1980; Featherstone *et al.*, 1981). It has been claimed that the microhardness test could detect enamel mineral loss earlier than replica electron-microscopic studies of the enamel surface (Koulourides,1968).

The disadvantages of this technique are that it is:-

1. Time-consuming.
2. Technique sensitive.
3. Destructive.
4. Measurements are not repeatable.
5. An indirect measurement, assumes a constant relationship between tissue resistance and mineral content (Koulourides *et al.*, 1968).
6. Less sensitive than Transverse Microradiography.

1.8.5 CHEMICAL ANALYSIS

This chemical method involves the dissolution of samples of hard tissue in acid, and the concentration of different elements is measured by means of atomic absorbtion spectrophotometry and other colorimetric techniques (ten Bosch and Angmar Mansson,1991).

Different sampling techniques are used which involve microdrilling (Hallsworth *et al.*, 1973), sequential acid etching (Weatherell *et al.*, 1973), and abrasion (Weatherell *et al.*, 1985).

This is a very sensitive technique for detecting mineral change, but it gives no indication of mineral distribution in various regions and lesion depth. Besides that, it is time-consuming, destructive, and depends upon the method of sampling.

1.8.6 POLARISED LIGHT MICROSCOPY

This method makes use of optical properties of crystals and relates the change in velocity of light to the density of a tooth lesion. Underwood (1989) used this technique to evaluate a fluoride-exchanging resin as an orthodontic adhesive.

When a beam of unpolarised light is passed through the crystal or a birefrigent material, it splits into two plane-polarised rays, vibrating at right angles to each other and exhibiting different refractive indices (Ten Bosch and Angmar-Mansson, 1991).

Enamel is made up of not only mineral and organic constituents, however, but also minute spaces. With net mineral loss during the caries process and lesion formation, there is an increase in the total volume of these spaces. The existence of pores within the crystalline structure contributes to the formation of form-birefringence. These pores can imbibe various media (e.g. water, naphthalene and Thoulet's solution) with different refractive indices, thus, changing the form-birefringence. The difference between intrinsic birefringence and form-birefringence can then be used to determine the mineral porosity

(Shellis and Poole, 1985). However, there are limitations to this technique which are listed as follows:

1. It does not provide quantitative measurement of mineral content.
2. Only lesion depth can be determined accurately by this means.
3. It assumes that the imbibing medium reaches even the smallest pores of the lesions.
4. It assumes that the crystallite optical axes are in the same plane as that of the tooth section.
5. It assumes that there are no pores so large that they do not contribute to form-birefringence (Shellis and Poole, 1985)
6. The use of imbibition media may affect the subsequent ability of the lesion to remineralise; hence serial measurement cannot be performed.

1.8.7 LIGHT SCATTERING

Scattering of light within a lesion makes incipient lesions look whiter than surrounding sound enamel. This is presumably due to the fact that the remaining small mineral particles in the lesion are embedded in water rather than in mineral-rich sound enamel which has a different refractive index (ten Bosch and Angmar-Mansson,1991).

This method was first developed by Borsboom and Ten Bosch in 1982 and was used by Ten Bosch *et al.* (1984) to quantify *in vitro* caries in flat, abraded slabs of enamel.

The optical caries monitor employs this working principle, providing an interesting and potential method of quantifying demineralisation of free smooth surfaces. However, its potential clinical use still requires testing and validation (Longbottom and Pitts, 1990).

1.8.8 ENDOSCOPICALLY VIEWED FILTERED FLUORESCENCE (EFF)

This technique utilises the fluorescence of enamel that occurs when it is illuminated with blue light in the wavelength range of 400 to 500 nm, emitted from a dental curing light. The tooth is viewed through a specific, broad-band Wratten-Gelatin filter, and white spot lesions appear darker than the surrounding sound enamel (Longbottom and Pitts, 1990).

Endoscopes allow a magnified image of the caries lesion to be viewed and this greatly aids clinical caries diagnosis. This technique has the advantages of having a wide viewing angle, access to otherwise difficult areas and manoeuverability of the endoscope. It allows small caries lesions, which would normally be clinically undetectable, to be visualised and would be very useful in detecting initial lesions *in vivo*, as these have the potential to remineralise.

1.8.9 SCANNING ELECTRON MICROSCOPY

Scanning Electron Microscopy has been used to assess the early caries lesion. Artun and Thylstrup (1986) used this technique to evaluate surface changes of incipient caries lesions after debonding. This involves coating the specimen with a special material, placing it in a vacuum and examining it under high magnification.

Ingram and Fejerskov (1986) used this technique to examine the differences in surface morphology of the early enamel lesion, and came to the conclusion that there were no measurable differences in the surface morphology between normal and carious enamel. This technique is, however, regarded as restrictive as well as destructive (ten Bosch and Angmar-Mansson ,1991).

1.8.10 IODINE PERMEABILITY /PENETRATION

This technique involves the estimation of the amount of iodide absorbed into the porous caries lesion by the use of an iodide-specific electrode.

Firstly, iodide fills up the porous part of the lesion and is then recovered by back diffusion into a known volume of water and quantitatively determined using an iodide-specific electrode. However, the reliability of the test may be affected by surface zone pore blockage, especially if used *in vivo* or *in situ* (Arends and ten Bosch, 1992).

1.8.11 SUMMARY

In vitro quantitative assessment of caries lesions can be performed accurately by transverse microradiography (Arends and ten Bosch, 1992) . The process is repeatable, and serial measurements are possible. Both mineral profile and lesion depth can be assessed accurately. Of the different methods discussed in this section, microradiography /microdensitometry seem to be the most comprehensive and reliable methods currently available for the study of mineral profile in the early caries lesion.

CHAPTER 2

MATERIALS AND METHODS

2.1 INTRODUCTION

This project consisted of both clinical and laboratory procedures. Details of the clinical procedures will be described from the time patients were selected, until they were discharged from the study. The laboratory procedures consisted of storing, processing and analyses of materials collected from the clinical trial. Saliva, plaque and urine samples were collected for the analysis of fluoride content while extracted teeth were examined for decalcification and associated mineral loss. Only one operator, (C.C), was involved in carrying out the entire programme of work.

2.2 PRE-CLINICAL PROCEDURES

2.2.1 ETHICAL APPROVAL

The *in vivo* study involved procedures that otherwise would not have been carried out as part of an orthodontic treatment plan. In order to protect the patients' right and assure that the patients' general and oral health were not compromised in any way, ethical approval was sought, and was granted in December 1994 (Appendix I).

2.2.2 PARENTAL CONSENT

As 96% of the subjects involved in this study were below the legal consenting age of sixteen years, the objectives of this clinical study together with the clinical procedures were explained to both the prospective patients and their

parent(s). The materials involved in the study were shown to the subjects. If they decided to participate, an explanatory note, together with a consent form, were given to the parent(s) to complete (Appendix II and III).

2.2.3 STUDY DESIGN

The cariostatic properties and fluoride-releasing ability of two resin-modified glass ionomer cements (Vitremer and Dyract) were compared to those of a control material (Right-On). Local and systemic uptake of fluoride were assessed by measuring the amount of fluoride in saliva, plaque and urine. The cariostatic properties of the test materials were assessed macroscopically, noting the presence or absence of decalcification in the vicinity of the bonded brackets and microscopically using microradiography and microdensitometry.

One of the two test materials was allocated to each subject at random. A half mouth technique was used for bonding - i.e. if upper premolars were to be extracted as part of the orthodontic treatment plan, a bracket was bonded on an upper right premolar with a test material, and a bracket was bonded with the control material on the opposite (upper left) premolar. If four premolars in the same mouth were to be bonded, bonding was undertaken using the Battenburg design - i.e. upper right/ lower left sites bonded with the test material and upper left/ lower right sites bonded with the control material. Extractions took place four weeks after bonding. All test and control teeth were extracted at the same visit. Details of the allocation of test and control materials were recorded in each patients case notes and were entered also on a data collection form (Appendix IV).

2.2.4. PATIENT SELECTION

Specific criteria

A total of 26 subjects participated in this study. They were either attending for orthodontic treatment at Glasgow Dental Hospital or at a general practice in Hamilton. Only those subjects whose treatment plan included extraction of 2 or 4 premolars were suitable for this clinical study. Subjects had no pre-existing glass ionomer restorations present in their mouth and were asked to have none placed throughout the duration of this study. Only intact, caries free premolar teeth were assessed as suitable for inclusion in this study. Any premolar tooth with buccal enamel surface discolouration, including any idiopathic enamel opacities, was excluded from the study.

General requirement

It is well recognised that orthodontic treatment can be detrimental to oral health if oral hygiene is inadequate. All subjects who participated in this study were subjectively assessed as having good oral hygiene and were well-motivated. All subjects were from in and around the Glasgow area where the drinking water has a fluoride concentration of 0.03ppm. The whole clinical trial lasted for eight weeks. Both the subjects and the parent(s) were enthusiastic and willing to adhere strightly to the study protocol.

2.3 CLINICAL PROCEDURES

2.3.1 CLINICAL MATERIALS

NON-FLUORIDE TOOTHPASTE

All subjects were issued with a 125ml non-fluoride toothpaste (Boots, Nottingham , U.K.), the composition of which may be found in Appendix

V. This non-fluoride toothpaste (Fig. 2.1) was to be used for a period of eight weeks in place of any other paste. This included the four week 'wash out' period and the four week trial period.

BRACKETS

The brackets (Fig. 2.2) used in this trial were . 022" pre-adjusted edgewise stainless steel upper and lower premolar brackets with hooks (Advant-Edge, Roth, TP Orthodontics Inc.). Occlusal tie wings were removed prior to placement to prevent possible debonding from occlusal loading (Fig. 2.3).

BONDING MATERIALS

The bonding materials used in this trial were:

Test	*Vitremer*	a tri-cured resin-modified glass ionomer cement (3M, USA)
	Dyract	a dual-cured resin-modified glass ionomer cement (Dentsply,UK)
Control	*Right-On*	a no-mix resin adhesive (T.P. Orthodontics, USA)

Vitremer (Fig. 2.4) is a tri-cured resin-modified glass ionomer cement. It is available as a hand mixed powder and liquid. This material has a unique curing system with three separate curing mechanisms. The first two curing mechanisms involve light cure of the resin and the chemical cure of the ionomer; the third curing mechanism involves chemical curing of the resin component. The manufacturer recommended that no acid etching is required for bonding.

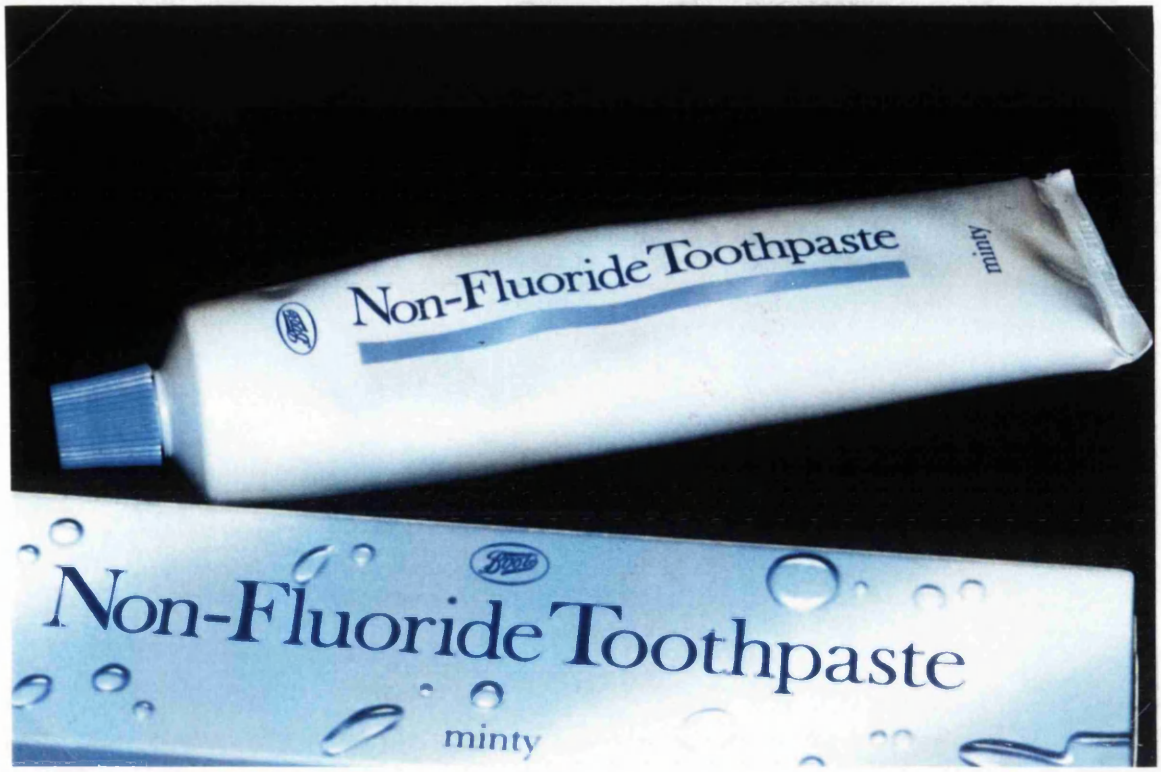


Fig. 2.1 A 125ml tube of non-fluoride toothpaste (Boots, UK)

Fig. 2.2 .022" pre-adjusted edgewise stainless steel brackets (Advant-Edge TP orthodontics, Inc.)

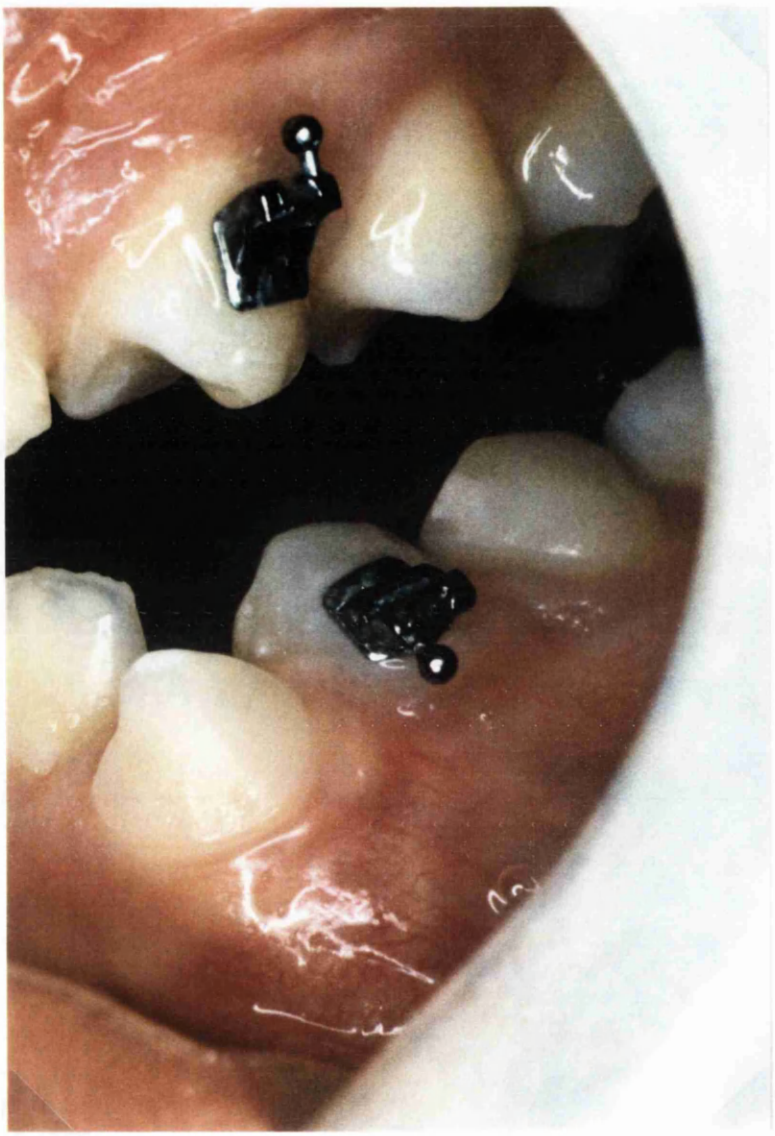


Fig. 2.3 Modified 0.022" pre-adjusted stainless steel upper and lower premolars brackets *in situ* with occlusal tie wings removed

Dyract (Fig. 2.5) is a dual-cured resin-modified glass ionomer cement. It is dispensed from a capsule and no mixing is involved. Its setting reaction is initiated by visible light, but full setting can be achieved through a dark reaction. The manufacturer recommended acid-etching for 30 seconds with 37 percent phosphoric acid.

Right-On (Fig. 2.6) is a no-mix resin adhesive. The activator is in the liquid and is applied to both the bracket base and to the enamel surface while the resin is dispensed from a capsule. The setting reaction is initiated when the resin contacts the initiator. Acid etching with 37 percent phosphoric acid is required for bracket retention.

2.3.2 CLINICAL METHOD

VISIT 1 - INFORMATION AND INSTRUCTIONS

The objectives of the study and guidelines for participating in the study were explained at this visit. When both the subject and parent(s) were happy with the arrangement, a consent form was signed and an information note given.

Subjects were required not to use any form of fluoride supplement throughout the duration of this investigation. A non-fluoride tooth paste of 125ml was issued to replace the one they used daily. This toothpaste was to be used by the subject alone, starting from *Visit 1* until the end of the trial period. General advice on tooth brushing and diet was given to ensure all patients practiced good oral hygiene measures.

Subjects were also advised not to brush their teeth for 24 hours before their next appointment. This temporary, one day, restraint from oral hygiene

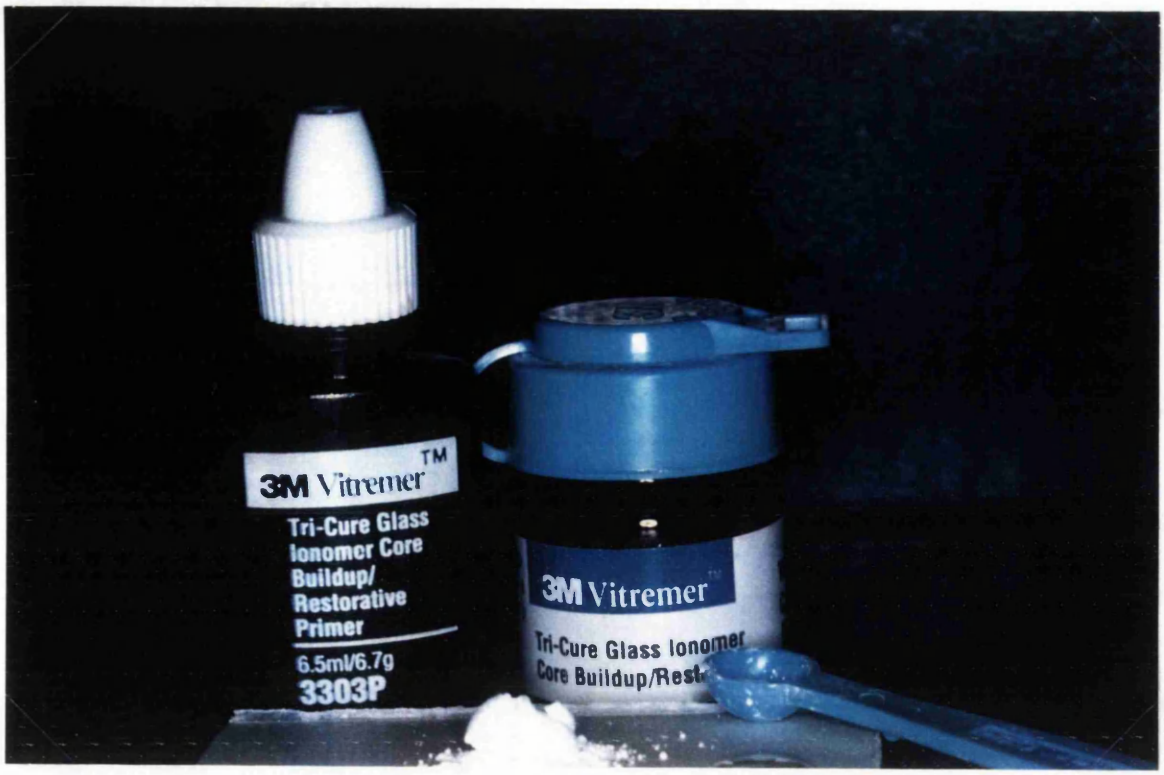


Fig. 2.4 Vitremer - powder and liquid for hand mixing

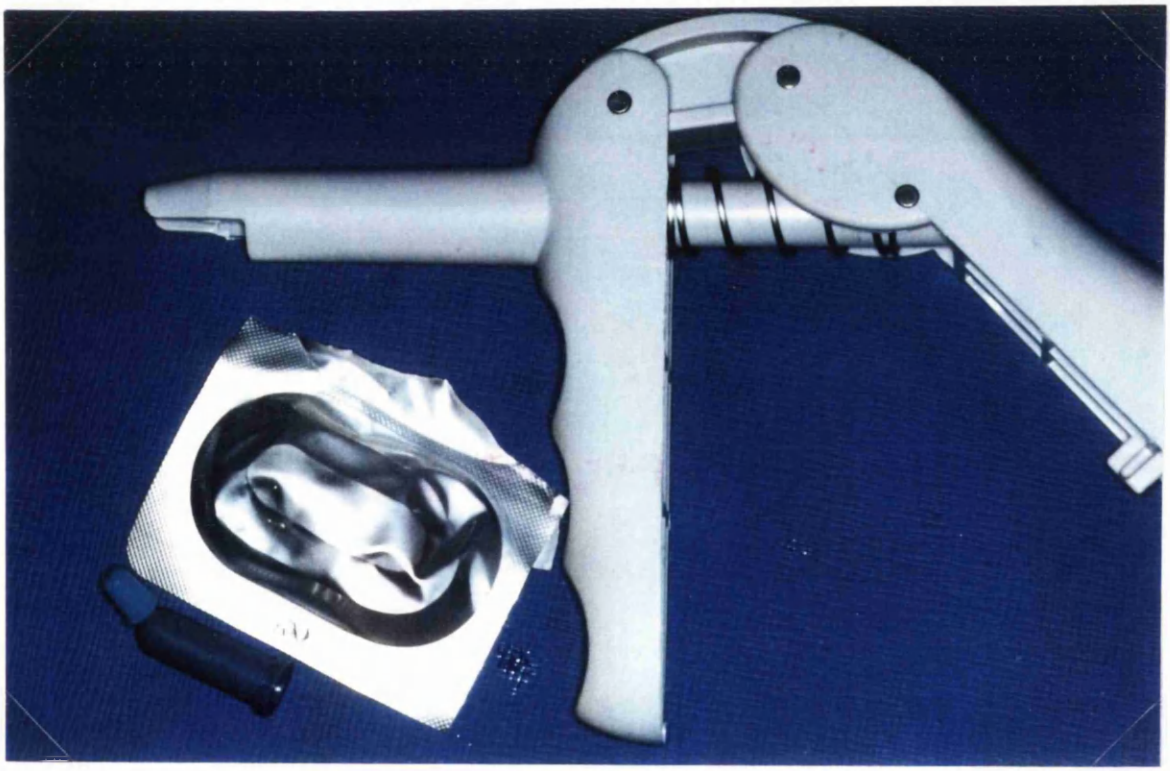


Fig. 2.5 Dyract - cement encapsulated

Fig. 2.6 Right-On - liquid etching solution, liquid adhesive activator and encapsulated adhesive.

measures before *Visit 2* would allow minor amounts of plaque to accumulate on the enamel surface for sampling.

A 100ml specimen container was given to each subject to take home. They were asked to collect an urine sample on the morning of their next appointment and bring it to the Department for collection. An appointment was arranged for 4 weeks later for bracket bonding.

VISIT 2 - SALIVA, PLAQUE AND URINE SAMPLING -TOOTH PREPARATION AND BONDING

Saliva, plaque and urine samples were collected, as described below, and stored at a temperature of $-20^{0}C$ for analysis in the Oral Science Unit at a later date.

SALIVA SAMPLING

Unstimulated saliva was collected from each subject before bonding. The patient was allowed to rest for 10 minutes with no stimulation to salivary flow. They were then asked to expectorate into a 5ml sampling container over a period of 5 minutes.

PLAQUE SAMPLING

Plaque samples were collected from the buccal surface of the pre-molars using sterile 100mm micro-spatulas (Chataway design). Subjects were asked to swallow before collection, so that salivary contamination was minimised. Any areas of obvious food debris were avoided. Samples from each site were placed separately into pre-weighed labelled 1.5 ml capped polypropylene micro-centrifuge tubes. Latex rubber gloves were always worn when handling

the tubes. Samples were weighed (Sartorius Analytic Balance A120S) shortly afterwards and weight estimated to the nearest 0.1 mg.

URINE SAMPLING

The urine sample was collected, checked and labelled before being stored at - 20^0C. Another 100ml specimen container was given to each subject to take home. They were asked to give an urine sample on the morning of their next appointment and bring it to the appointment for collection.

BRACKET BONDING

The pattern in which the bonding materials were allocated has already been described in section 2.2.3 (study design). Prophylaxis, acid etching and bonding procedures were carried out as follows:

(1) Prophylaxis

Prophylaxis of the buccal surface of the teeth was preformed with a rubber cup and the prescribed non-fluoride tooth paste. After 10 seconds of polishing, the tooth surface was washed with water for 10 seconds. Moisture control was maintained throughout with high volume aspiration and cotton wool rolls. The tooth surface was then dried thoroughly with a stream of warm air from an electric hair dryer unit for approximately 5 to 10 seconds.

(2) Acid-etching

Premolar teeth bonded with Dyract and Right-On were etched before bonding, whilst those bonded with Vitremer were not etched in accordance with the manufacturers' instructions. Acid etching was carried out using a gel etchant (37% phosphoric acid) for 30 seconds. The etching gel was applied with a brush only to the site where the bracket was to be bonded. This minimized unnecessary demineralization of the enamel surface. The tooth surface was

washed with water for 30 sec and then dried by warm air from a hair dryer for roughly 10 seconds. The etched and dried tooth surface was protected from saliva contamination by holding the soft tissues away with cotton wool rolls and aspirator tips.

(3) Bonding

Each bracket was positioned in the centre of the buccal enamel surface along the long axis of each premolar and pressed firmly into place. A sharp probe was used to remove excess cement from the bracket margins. Brackets bonded with Dyract or Vitremer were then cured for 10 seconds from the incisal, gingival, mesial and distal aspects of the bracket base using a visible dental curing light (ESPE, UK). Once all the clinical procedures were completed, patients were given the usual instructions for fixed appliance care and instructed to brush twice daily using a non-fluoride toothpaste. A 100ml sampling container was issued for an urine sample to be taken early in the morning of the next appointment, which was scheduled for four weeks later. Subjects were asked to refrain from their usual oral hygiene measures for 24 hrs prior to their next appointment.

VISIT 3 -SALIVA, PLAQUE AND URINE SAMPLING
-EXTRACTIONS
-DISCHARGING PATIENT

SALIVA, PLAQUE AND URINE SAMPLING

Saliva, plaque and urine samples were collected as in *visit 2*. Having checked the patients' medical history, the bonded premolar teeth were extracted as described below.

EXTRACTIONS

Premolars were extracted under local anaesthesia (2% xylocaine) by one operator (C. C). It was essential that the clinical crown remained intact and the enamel surface undamaged to allow proper microdensitometric assessments to be made. To avoid possible damage, a non-traumatic extraction technique was used. The tooth was first loosened from the socket by slightly expanding it with an elevator. A cotton wool roll was then placed over the occlusal, buccal and lingual surfaces of the tooth before a pair of dental extraction forceps was applied. The presence of the cotton wool roll was essential to minimize enamel crazing during the extraction procedure. Once the tooth was delivered, with the bonded bracket *in situ*, it was rinsed with deionised water and then stored in a labelled 10ml container filled with a 2% thymol solution .

DISCHARGING PATIENT

After the extractions, each patient was advised to resume their normal oral hygiene procedures with the use of a fluoridated toothpaste and arrangements were made for continuation of their orthodontic treatment.

2.4 LABORATORY PROCEDURES

2.4.1 DEBONDING AND PHOTOGRAPHIC RECORD OF EXTRACTED TEETH

Before photographic records were taken, bonded brackets were carefully removed by using a pair of universal forceps without damaging the enamel surface. No attempt was made to remove any residual bonding material left on the enamel surface.

Colour transparencies were taken by mounting a single lens reflect camera Nikon F3 on a light microscope (Carl, Zeiss.) and photographic records were obtained under the following standardised conditions:

1. The tooth surface was dried.
2. Uniform background illumination.
3. Film speed : ASA 100
4. Exposure : 10 sec
5. Magnification : 4X

2.4.2 ASSESSMENT OF DECALCIFICATION OF EXTRACTED TEETH

A macroscopic visual assessment of each extracted tooth after debonding was made firstly to check for demineralization and cavitation. Then the condition of the buccal enamel surface was recorded on colour transparency film. When the film had been developed, the colour slides were coded, randomly arranged and projected in a darkened room. A calibration exercise was undertaken using a random sample of ten slides before the assessment of decalcification was made. Two examiners who were familiar with, and who had been previously experienced in the use of the von der Fehr Caries Index scored each transparency.

Examiners had 20 seconds only to score each transparency. All 96 slides were scored by both examiners on two separate occasions, two weeks apart, using the criteria of von der Fehr, (1961) with slight modification. Adopting this protocol allowed intra- and inter- examiner reliability to be examined.

Scores were awarded as shown in the following table:

Table 2.1 modified von der Fehr Caries Index System

Score	Criteria
0	surface appears intact
1	limited greyish tinge with or without accentuated perikymata
2	perikymata well accentuated, in some areas confluening into greyish-white spots
3	pronounced white demineralization (i.e. frank white spot lesion)
4	cavitation

2.4.3 SECTIONING OF EXTRACTED TEETH

Once satisfactory photographic records of the extracted teeth were taken they were prepared for microradiography and microdensitometry.

The root(s) of each extracted tooth was removed with a microsaw. Each tooth was cut 2mm apical to the cervical margin of the crown. The tooth was positioned on a small metal arm with the buccal surface facing up towards the cutting edge of the blade, and orientated at 45 degrees. Tan wax was softened in a bunsen and allowed to flow onto the tooth surface to achieve close adaptation all around. The clinical crown of the tooth was then fully embedded and supported by tan wax. This was carried out to secure the tooth position before it was mounted on the cutting machine and also to minimize possible fracture of the enamel surface in the cutting process.

The tooth was sectioned using a Microslice 2 (Metals Research Ltd., Cambridge, England) as shown in Figure 2.7.1 and Figure 2.7.2. Sections were cut by the diamond coated rotating wheel at high speed. A coolant

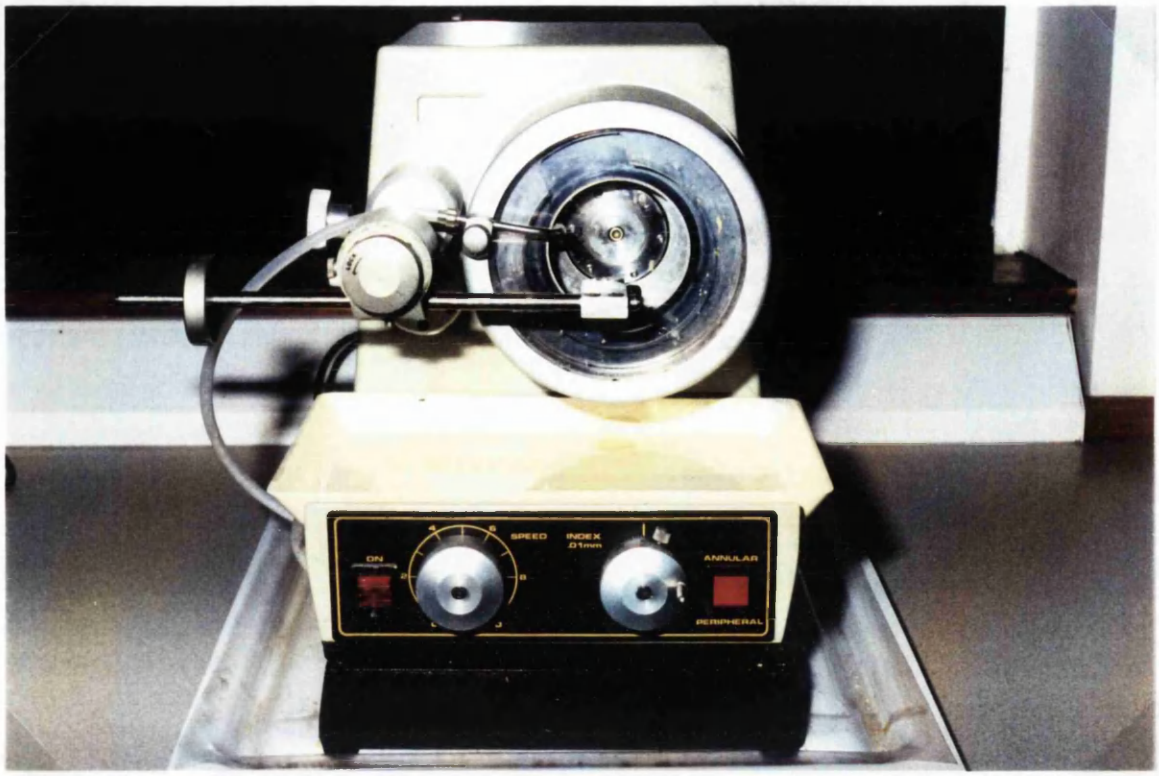


Fig. 2.7.1 The Microslice 2 cutting machine

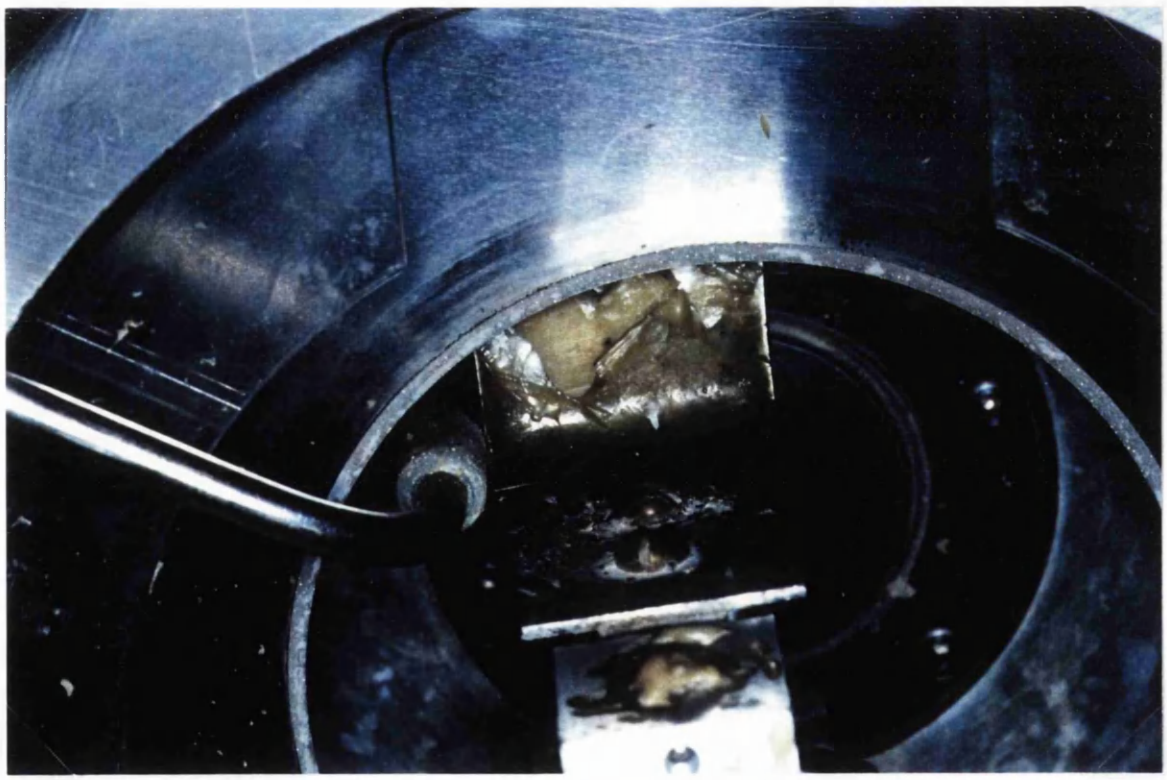


Fig. 2.7.2 A close-up view of a tooth section embedded in tan wax (behind the diamond coated grinding wheel) and mounted on Microslice 2.

was used throughout the cutting process to prevent clogging of the cutting blade and over heating of both blade and specimen.

The first cut was made halving the tooth bucco-lingually. The remaining tooth crown which was still mounted on the chunk was then dried and examined. A small piece of glass slide was applied to fully cover the cut tooth surface, and the slide was retained by using "Loctite 454 " adhesive (Loctite, U.K., Ltd., Welwyn Garden City, England). This was used to support the thin section and prevent it from fracturing.

Once the adhesive was fully set after 1 min, the Microslice was adjusted to cut the next section at approximately 250 μm. The buccal surface of this thin section covered the area where the bracket was bonded and also the enamel surface both gingival and occlusal to it. This is shown in Figure 2.8.

The cut slide was stored in 2% thymol again before the supporting glass slide was debonded. Adhesive on the glass slide was removed by soaking in Acetone for 15 min. The cut slide was then rinsed in alcohol to remove the acetone and finally washed in deionised water . The debonded section was finally halved mesio-distally keeping only the buccal section (the surface previously bonded with one of the three bonding agents) for further analysis.

2.4.4 LAPPING OF SECTIONED TEETH

As a tooth section of 250μm thickness would be too thick for microradiographic and microdensitometric analysis, it was necessary to reduce it. Lapping down of the section was done by using a slurry of carborundum powder (particle size - 0.3μm - Raymond A . Lamb, London, England) and water on a ground glass plate as shown in Fig. 2.9. The tooth section was placed onto the moistened surface of a heavy brass plate

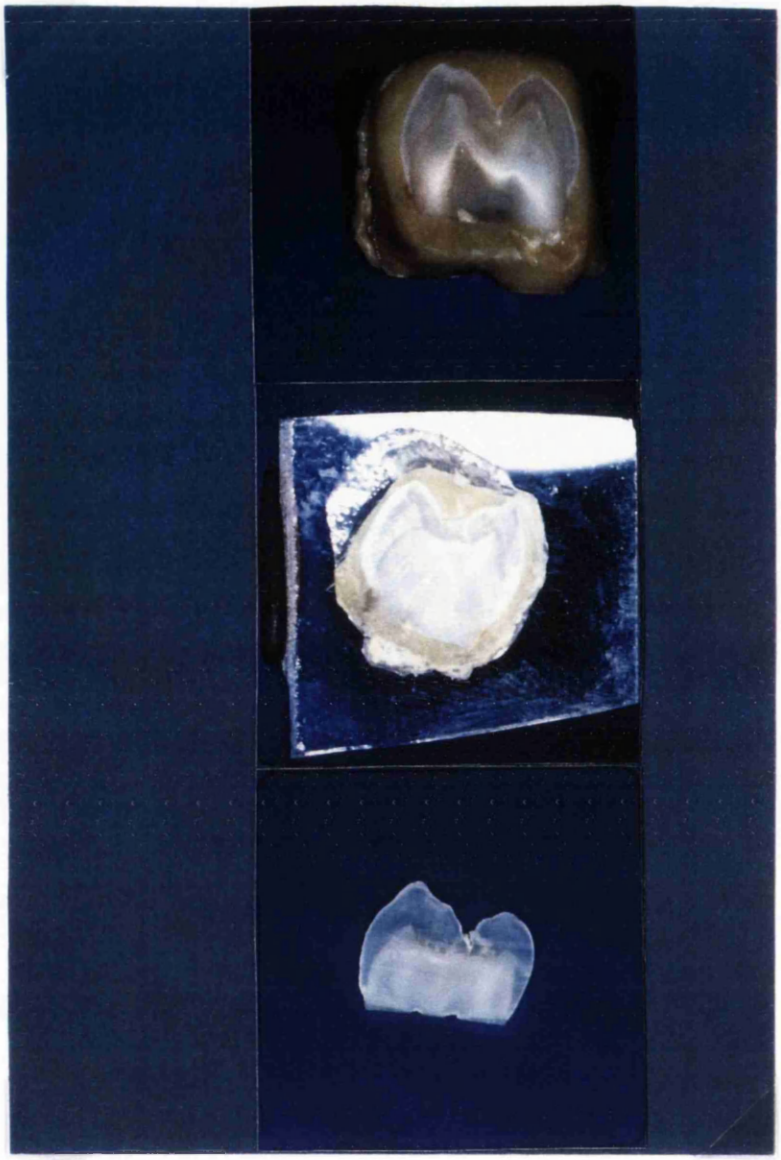


Fig. 2.8 Cross-sectional views of a sectioned tooth
Top view: embedded in tan wax
Middle view: at approximately 250*u*m supported by a thin glass slide
Bottom view: debonded section at 250*u*m

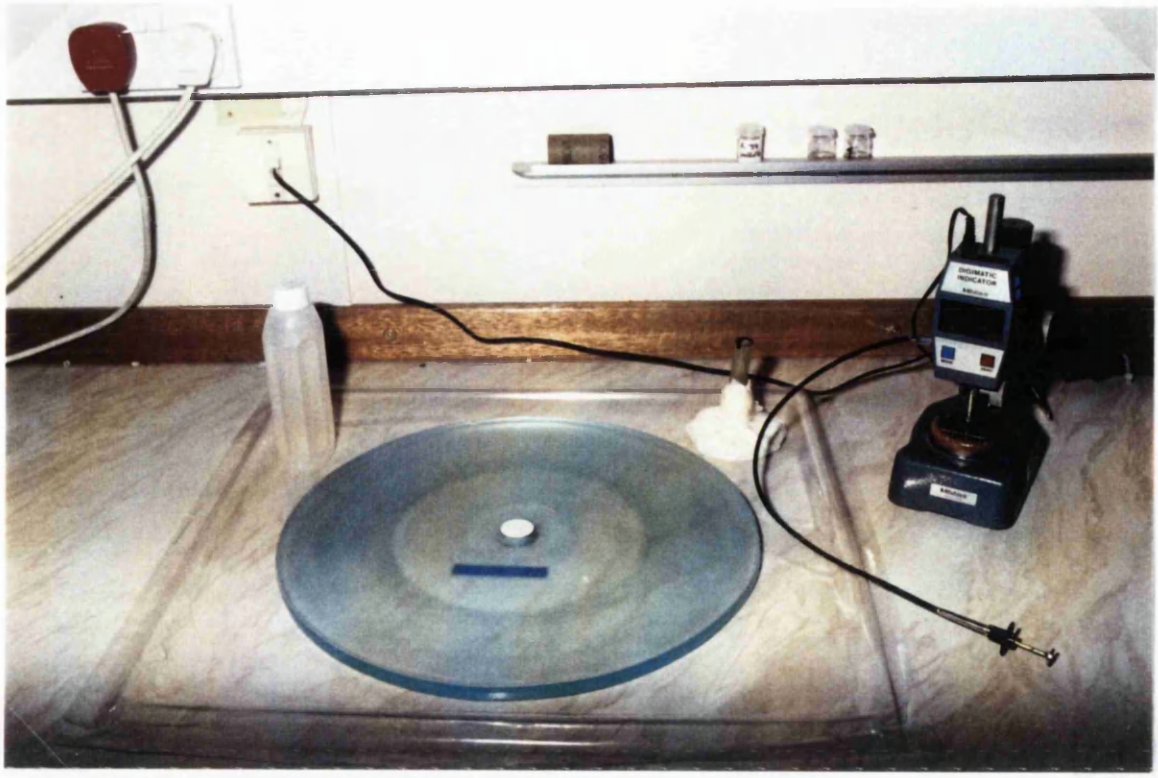


Fig. 2.9 1. Ground glass plate used for lapping down tooth section (centre)
2. Digital micrometer (right)

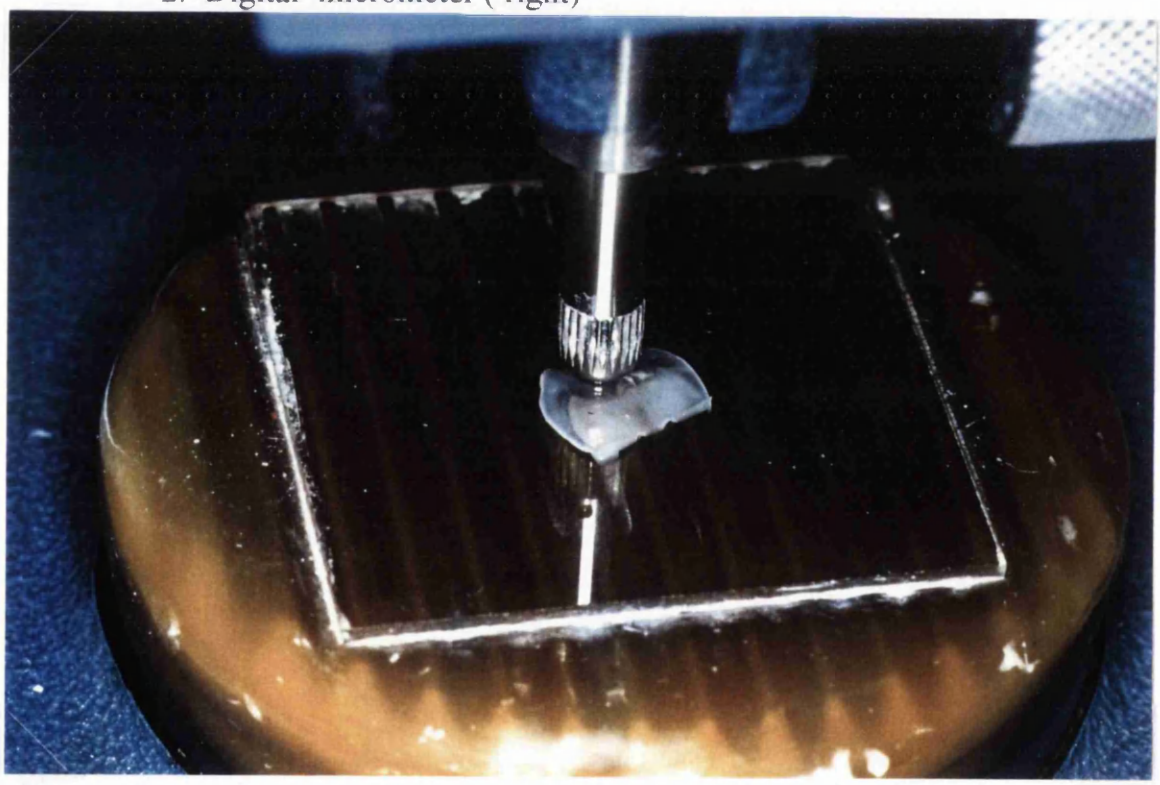


Fig. 2.10 A close-up view of measuring a tooth section by using a digital micrometer.

covered in damp gauze. This heavy brass plate with the tooth section mounted on it was rotated slowly round the glass plate in a circular motion . Both the cut surfaces of the tooth section were ground in turn, making sure a uniform thickness and a complete smooth surface were achieved on both sides. The section thickness was checked occassionally during the lapping process by using a digital micrometer (Mitutoyo, Tokyo, Japan) as illustrated in Fig. 2.10. Measurements of thickness were made in the cross-section 1mm apart from each other, from the occlusal to the gingival aspect of the enamel surface. The whole lapping process usually took 10 min to complete.

Once the appropriate thickness was achieved (approximately 130-140μm in thickness), the ground section was marked with a graphite pencil (for easier identification) and stored in a 2% thymol solution in readiness for microradiography.

2.4.5 MICRORADIOGRAPHY

The tooth sections of required thickness were mounted in clingfilm and placed over a microradiographic plate with an aluminium stepwedge placed alongside the sections for later calibration. These were then placed inside light tight plate holders ready for exposure.

Previous work (Creanor, 1987) showed an unacceptable variation in X-ray beam homogeneity from the top to the bottom of the plate, while the variation was found to be less than 1 % along the x-axis. To minimize variation in beam homogeneity, the aluminium stepwedge was positioned along the y-axis. The microradiographs of the sections and an aluminum stepwedge (Fig. 2.11) were taken with a 20 minute exposure on the plate (Kodak high resolution plates [Type 1 A] Eastman Kodak Company, Rochester, New York, USA) by a Cu(Kα) x-ray source (Marconi TX 12) operating at 20kV and 30mA at a

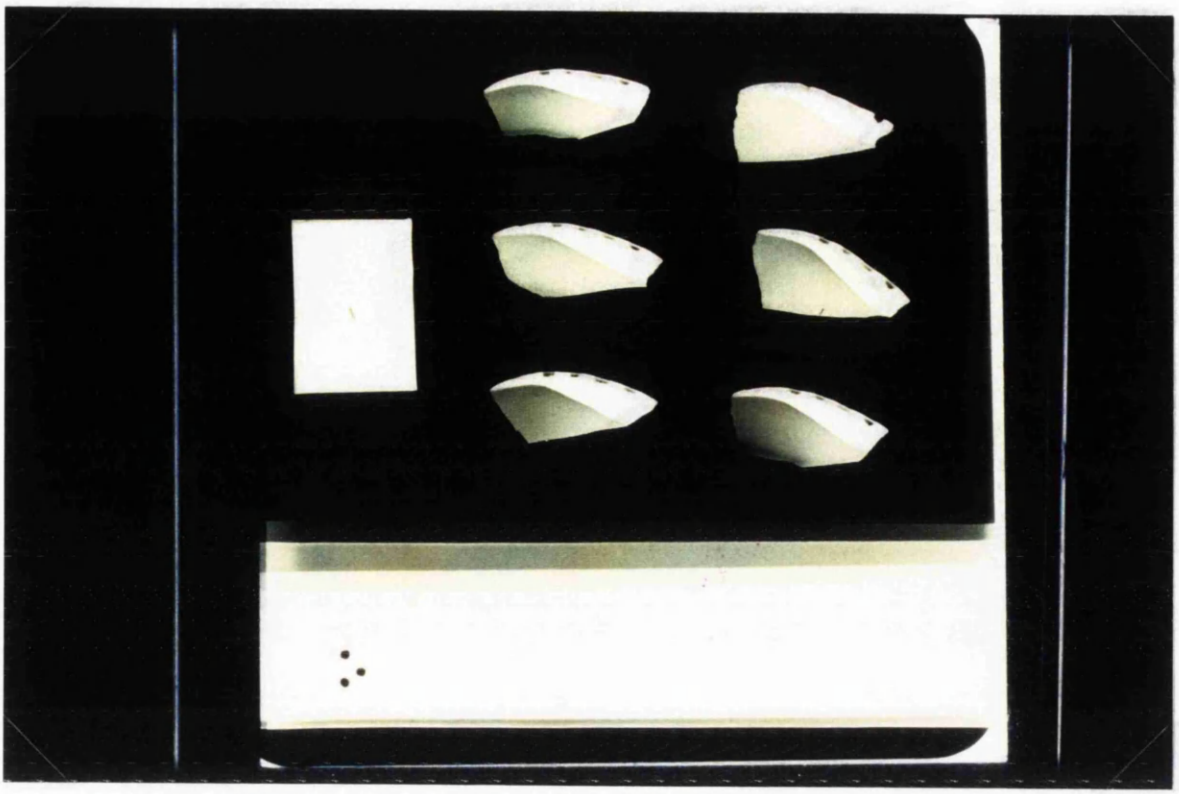


Fig. 2.11 Microradiographs of the tooth sections (top) and an aluminum stepwedge (bottom)

Fig. 2.12 X-ray machine, Marconi TX 12 (DIFFRACTIS 582)

focus-source distance of 300mm (Figure 2.12). This permitted micro-densitometric measurements of the aluminium wedge to be made at the same level as the measurements of the lesion, thus benefitting from the x-axis beam homogeneity.

The microradiographs were then developed using standardised techniques. Subject to satisfactory exposure, the microradiographs could then be used to examine for caries lesions and analyse the mineral profile of lesions by means of microdensitometry.

2.4.6 MICRODENSITOMETRY

The microradiograph was placed on the specimen stage of a microscope (Fig. 2.13), Leitz Ortholux II, which was illuminated by a uniform stablised light source. The image (Fig. 2.14) was recorded by a video camera (ASACA Corporation type 700BE) and transmitted to an analyser unit (Leitz Image Analyser). This unit was controlled by a Z8002 microprocessor which digitised the video signal from the camera into 256 grey levels with a resolution of 256 x 256 pixels. Potentiometers on the front panel of the image analyser enabled the unit to be set up so that the 256 grey levels covered the range of optical, radiographic densities of the material to be analysed. The digitised image (Fig. 2.15) was then transferred to a computer for further analysis and data storage. The software for the computer and the Leitz Image Analyser were developed by Brian Reece Scientific Limited (Berkshire, U.K).

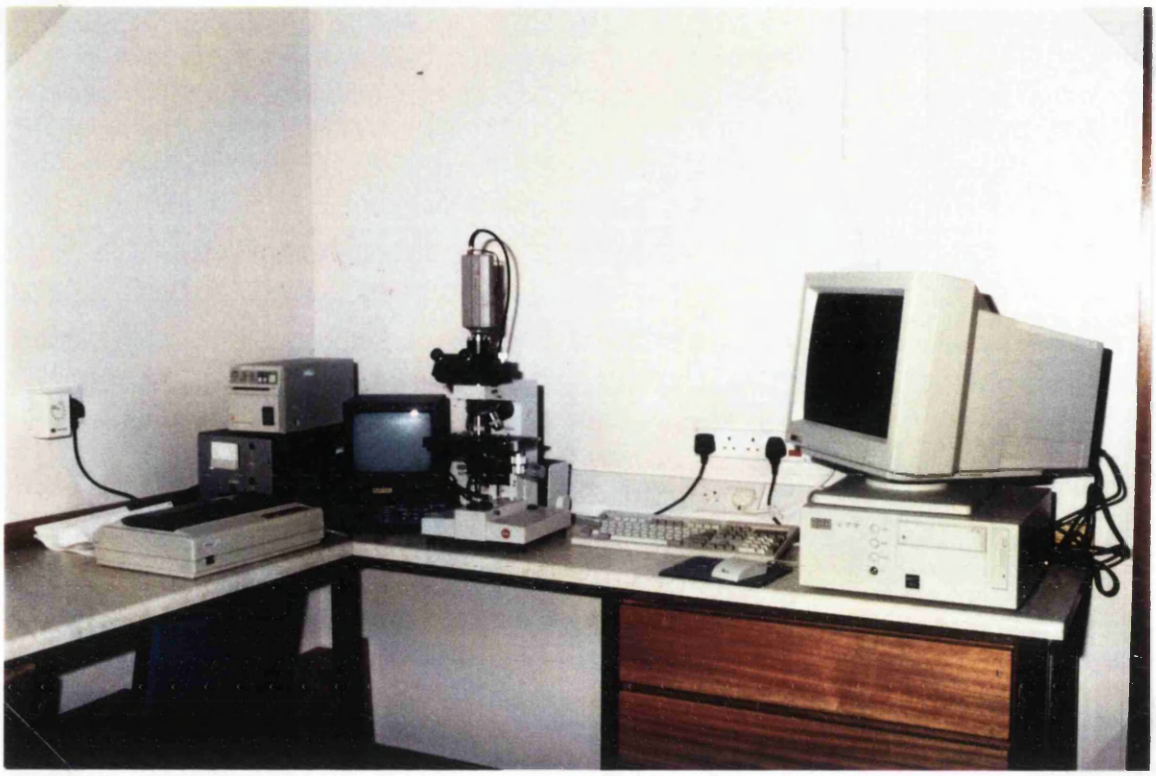


Fig. 2.13 centre - microscope, Leitz Ortholux II and video camera (ASACA Corporation, type 700BE)
left - analyser unit, Leitz Image Analyser
right - computer monitor, Bager

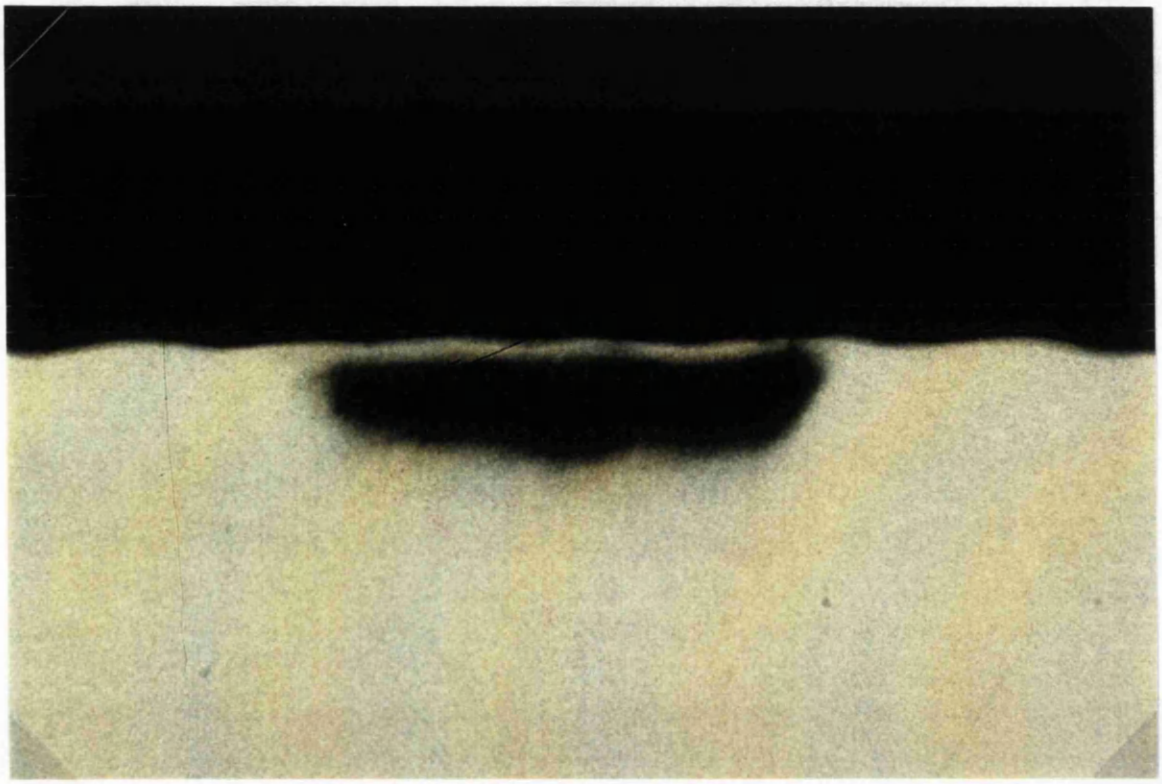


Fig. 2.14 Radiographic image of a subsurface caries lesion

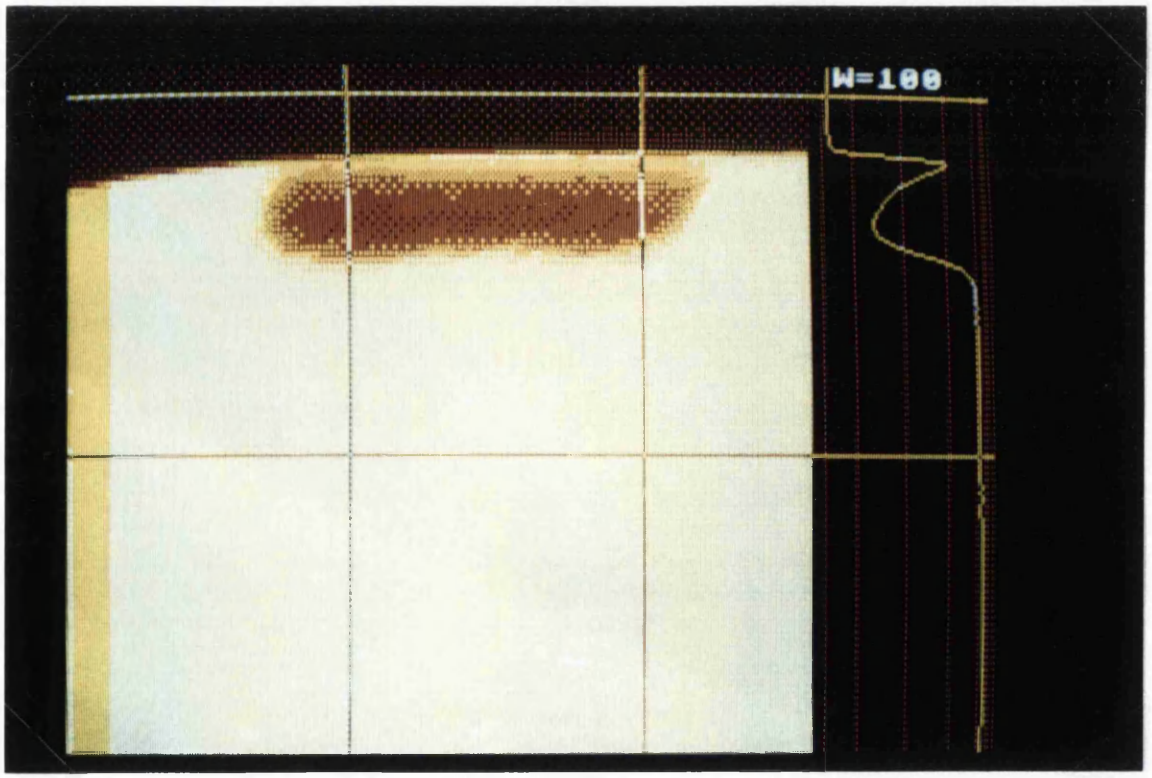


Fig. 2.15 Digitised image of a subsurface caries lesion

Calibration of the system using an aluminium step wedge

The microradiographic plate was initially positioned so that the thickest part of the wedge was in the field of view. The camera was then saturated with light and the potentiometer adjusted to record a value of approximately 250 grey levels. The camera was then blanked out so that no light was detected. The potentiometer was adjusted to record a reading between 6 and 9 grey levels. Finally, the light source was adjusted so that the camera recorded the light passing through the thickest part of the wedge at about 241 grey levels. The plate was repositioned to record the level of light passing through the remaining 6 portions of the aluminium step wedge in turn.

Defining area of interest on the radiograph

The radiograph was positioned to view the area of the particular lesion of interest on the same horizontal axis as the scan taken from the step wedge. The camera was rotated so that the surface zone of the lesion was horizontal on the camera monitor. The image was then digitised and displayed on the computer monitor. The area of interest (AOI) was then chosen by the mouse. An average microdensitometric profile could then be calculated in this region.

Lesion measurement

Once the AOI was defined and subsequently scanned, an averaged optical density-pixel distance graph was displayed on the monitor. Further analysis was carried out and the results were converted from optical density and pixel number to mineral content and real distance. A new mineral content-distance graph was produced and the results saved on disc after obtaining a print out.

Measured parameters

1. All lesion tracings were normalised to a sound enamel mineral content of 80% prior to data handling. This procedure reduces the influence of second order effects, such as accidental marks on the radiographic plate, on the calculated data. The measured value for normal enamel varies in the literature from 78% to 87% by volume (Groeneveld,1974). Therefore, the value of 80% was considered to be within this accepted range.
2. The minimum lesion mineral content was taken as the lowest point on the mineral profile deep to the surface zone.
3. The per cent volume mineral content of the surface zone was taken at the maximum mineral content of the surface layer.
4. The lesion depth, Ld, was calculated as the distance from the 20% mineral content of the initial slope to an arbitary cut-off point at approximately 95% of the value of normal enamel. This arbitrary cut-off point was selected because of the unreliability of determining depths in lesions where the mineral content approaches that of sound enamel (Mallon & Mellberg,1992).
5. The integrated mineral loss (Δz) was calculated as the area above the profile from a point at the 20% volume mineral level on the initial slope to a point (S) in sound enamel. Providing the section is planoparallel, the positioning of point S in sound enamel is unimportant.

2.4.7 QUANTIFICATION OF FLUORIDE IN SALIVA

The ionanalyser (Expandable Ion Analyser EA 940, Orion Research, Fig. 2.16), connected to a fluoride ion selective electrode (Russell Fluoride Ion Selective Electrode ISE 96-6099, Fig. 2.17), was checked by measuring the electrode slope (Appendix VI) before any measurement could be made. A low level fluoride calibration curve was then obtained (Appendix VII).

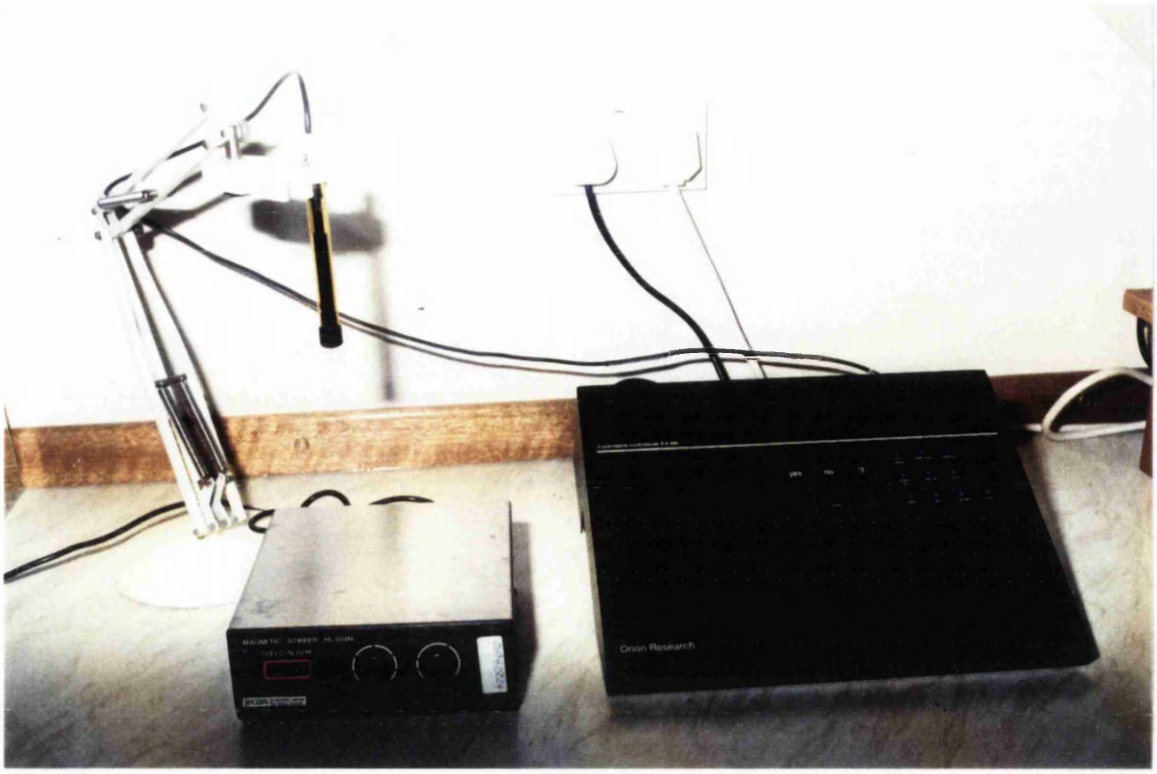


Fig. 2.16 Expandable Ion Analyser EA 940, Orion Research

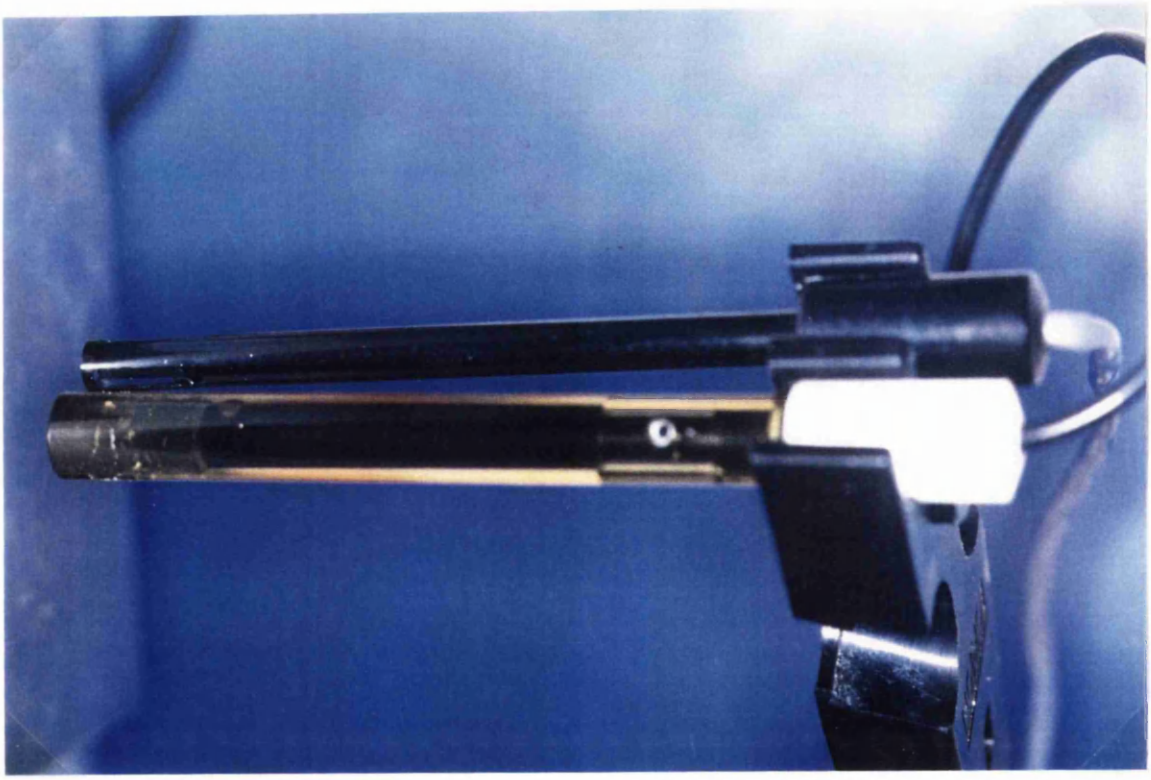


Fig. 2.17 Russell Fluoride Ion Selective Electrode (ISE 96-6099)

Saliva samples were brought to room temperature by leaving them out of the freezer for three hours. 200 μl of the saliva sample were vortexed with 200μl of low level 'total ionic strength adjustment buffer' (TISAB, Appendix VIII) for 30 seconds until thoroughly mixed.

Mixing with TISAB solution served two functions:

1. This buffer adjusted the ionic strength of the sample and thus prevented drifting of potentiometric measurements by the electrode.
2. TISAB adjusted the pH of the suspension to between 5 and 5.5, producing standardised conditions under which fluoride is measured.

200μl of the mixture was then removed and placed into an 'Orion microsample dish'. Gentle mixing was provided by a stirrer driven by magnetic force. The microsample dish was covered while the electrode was allowed to stabilise for 5 minutes before mV measurements were recorded. The lanthanum fluoride membrane of the fluoride electrode was cleaned carefully and dried between measurements.

The mV readings were converted to parts per million fluoride from a low level calibration curve .

2.4.8 QUANTIFICATION OF FLUORIDE IN PLAQUE

Prior to the assessment of plaque fluoride, all the plaque samples were removed from the freezer and allowed to thaw to room temperature for 3 hours. The fluoride ion selective electrode was checked and a low level fluoride calibration curve was obtained.

200 μl of deionized water and 200 μl of low level TISAB were added to the defrosted plaque samples. The samples were then whirlmixed for about 30 sec using a Vortex Mixer until the mixture became completely homogenised.

200μl of the mixture were then removed and placed into an 'Orion microsample dish' by using an Oxford micropipette. The sample dish was positioned on a stirrer with a polystyrene block placed between the dish and the stirrer to prevent heat conducted from the stirrer to the solutions in the dish. A plastic coated magnet was then introduced into the sample dish and allowed to vibrate at a speed which allowed gentle mixing of the solution. A piece of cling film was used to seal round the dish and the electrode to prevent water loss by dehydration during mixing.

After 5 minutes equilibration, the millivolt reading was noted from the ionanalyser. The electrode was washed with deionized water, gently cleaned with a non-fluoride toothpaste and then rinsed throughly with deionized water again. This prevented a build-up of organic material between measurements which might affect the accuracy. Once the electrode was dried, it was ready for the analysis of the next sample.

The mV readings were later converted to ppm fluoride from a low level calibration curve by using a special computer programme designed by the Unit of Oral Sciences in Glasgow University. The true fluoride concentrations were then calculated (Appendix IX) and expressed as ppm fluoride per mg wet weight of plaque.

2.4.9 QUANTIFICATION OF FLUORIDE IN URINE

Fluoride in urine was analysed in a similar manner to that described previously for saliva and plaque, using the same technique and equipment.

The laboratory procedures were as follows:

1. Low level and high level fluoride calibration curves were prepared.
2. Urine samples were brought back to room temperature .
3. 200μl of urine were mixed well with 200μl of low level TISAB.
4. 200μl of the mixture were placed in a microsample dish.
5. Measurements (mV) in the Ionanalyser were taken after 5 min.
6. Each mV reading was converted to ppm of fluoride.

CHAPTER 3

EXPERIMENTAL FINDINGS AND STATISTICAL METHODS

3.1 CLINICAL PROCEDURES

3.1.1 VISIT 1

There were 26 patients involved in this study; 20 of the subjects were patients of the Glasgow Dental Hospital and the remaining 6 were patients from a Hamilton Dental Practice. Since they all came from areas, where the drinking water is not fluoridated, and they all adhered to the same study protocol, the data from both sources were combined for analyses. Details of the samples and teeth analysed are given in Table 3.1.

96 premolars (50 upper; 46 lower) were involved in this study. In 22 patients 4 premolars were involved in the study, while in 4 patients only 2 premolars were involved.

3.1.2 VISIT 2

Test materials were allocated at random; only one test material was used in each mouth. Details of the distribution of bonding materials in each quadrant of the mouth are shown in Table 3.2.

96 plaque samples, 26 saliva samples and 26 urine samples were collected on visit 2 for later fluoride analysis.

3.1.3 VISIT 3

A further 96 plaque samples, 26 saliva samples and 26 urine samples were collected at visit 3 from each of the 26 patients.

TABLE 3.1

The age and sex distribution, number of teeth involved in the maxilla and mandible, and Angle's classification of malocclusion in the study sample.

AGE (yrs)	MEAN	13.4
	RANGE	11.6 - 21.6
SEX	MALE (M)	11
	FEMALE (F)	15
	Total	26
TEETH	MAXILLARY	50
	MANDIBULAR	46
	Total	96
MALOCCLUSION (ANGLE'S CLASSIFICATION)	II (1)	19
	I	7

Table 3.2 Distribution of bonding materials in each quadrant of the mouth.

Quadrant	Material			Total
	Test		Control	
	Vitremer	Dyract	Right-On	
Upper Right	7	4	14	25
Upper Left	6	8	11	25
Lower Right	6	8	9	23
Lower Left	6	3	14	23
Total	25	23	48	96

Two brackets (2% of the total) were debonded before the end of the 4 week trial period; one had been bonded with Vitremer, while the other had been bonded with Right-On. The bracket bonded with Vitremer debonded a few hours before the end of the trial period and the tooth was included in the data for analysis as the bracket had already been in place for 30 days. The bracket retained with Right-On debonded just two days after visit 2 and the related tooth was excluded from the analysis. The failure rates of the three bonding materials in the four week trial period are shown in Table 3.3.

All the premolars that were bonded with brackets were extracted after a mean time of 30 (+/- 2) days as described in Chapter 2. The 96 extracted teeth were then stored in a 2% thymol solution.

3.2 LABORATORY PROCEDURES

3.2.1 DISTRIBUTION OF WHITE SPOT LESIONS

Macroscopic examination by one examiner (C.C.) of each of the 96 extracted teeth revealed ten teeth which exhibited marked white spot lesions or cavitation; all the others appeared to have an intact enamel surface. One month later, the colour transparencies of each tooth were coded and randomly arranged and projected onto a screen in a darkened room. Each transparency was scored for decalcification by two examiners independently under identical conditions on two separate occasions, two weeks apart. Scoring of decalcification was based on the modified von der Fehr Index System, the criteria of which were described in section 2.5.5. During the calibration exercise it became obvious that it was impossible to distinguish accurately between lesions scoring 1 or 2 (greyish discolouration) under the magnification imposed by projecting the transparencies. This difficulty was most likely due to photographic flashback and artefacts produced by the curvature of the buccal

Table 3.3 Frequency of bond failure with each of the bonding materials in each region of the mouth.

Quadrant				Total
	Material			
	Test		Control	
	Vitremer	Dyract	Right-On	
Upper Right	0	0	1	1(4%)
Upper Left	1	0	0	1(4%)
Lower Right	0	0	0	0
Lower Left	0	0	0	0
Total	1(4%)	0	1(2%)	2(2%)

surface of the tooth. For further assessment, the index was amended so that only scores 0, 3 and 4 were used. As the transparencies were scored by two examiners independently, the results of the reproducibility study will be discussed first. Advise on statistical methods was given by Mr. J. McColl and Mr. H. Gilmour of the Department of Statistics of the University of Glasgow.

With respect to intra-examiner reliability, examiner A was found to be more consistent than examiner B. The percentage reproducibility of examiner A and examiner B were 82% and 64% respectively. Mean inter-examiner reliability was 70% (56% on the first and 83% on the second occasion). This indicated that there was good agreement between the two examiners on the second occasion, even though examiner B was less consistent with himself than examiner A on the two occasions. It was decided therefore to base further analyses on examiner A's second observations. The results of the indirect visual assessment (examiner A occasion 2) are tabulated in Table 3.4 .

Each subject's scores of the experimental and control teeth were paired for analyses. Mean scores were calculated for control teeth and test teeth separately (Table 3.5). Statistical advice indicated that Kruskal-Wallis tests were the most appropriate analyses and these tests were then carried out to determine whether there was any significant difference between the experimental and control materials with respect to decalcification. As there was no significant difference between the two fluoride releasing test materials (Vitremer *v* Dyract) and the control materials (Right-On *v* Right-On), the test and control data were pooled separately for further analysis (Table 3.6).

Table 3.4
Score distribution for each material.

U=upper arch
L=lower arch

0= no white spot
3= frank white spot
4= cavitation

	Score			
	0	3	4	total
Vitremer U	0	0	0	13
L	0	0	0	12
U+L	25	0	0	25
Right-On U	0	3	0	13
L	0	1	0	12
U+L	21	4	0	25
Dyract U	0	0	0	12
L	1	0	1	11
U+L	22	0	1	23
Right-On U	0	2	0	12
L	0	2	1	11
U+L	18	4	1	23
total	86	8	2	96

Table 3.5
Mean scores of test and control teeth in the two experimental groups per subject.

Number of subjects in each group = 13
Number of teeth involved = 96

Vitremer group			Dyract group		
Subject	Test teeth mean score	Control teeth mean score	Subject	Test teeth mean score	Control teeth mean score
1	0	0	1	0	0
2	0	1.5	2	0	0
3	0	0	3	0	1.5
4	0	1.5	4	0	0
5	0	0	5	0	1.5
6	0	1.5	6	0	1.5
7	0	0	7	0	0
8	0	0	8	4	4
9	0	0	9	0	0
10	0	1.5	10	0	0
11	0	0	11	0	0
12	0	0	12	0	0
13	0	0	13	0	0

Table 3.6
Summary of statistical analysis of indirect visual assessments

Kruskal-Wallis test (adjusted for ties)	Number of patients	P-value
Vitremer vs Right-On	13	0.033
Dyract vs Right-On	13	0.179
Vitremer vs Dyract	26	0.318
Right-On vs Right-On	26	0.899
Test materials vs Control material	26	0.015

The findings were as follows:

1. None of the premolars bonded with Vitremer had frank white spot lesions (score 3) or cavitation (score 4), while 16% of the control teeth in the Vitremer group had scored 3.
2. One premolar (4%) in the Dyract group scored 4, and none scored 3 in contrast to 22% of the control teeth which scored 3 or 4.
3. Overall, 2% of premolars bonded with the fluoride-releasing materials in contrast to 19% of premolars bonded with the nonfluoride-releasing material developed early carious lesions or cavitation after four weeks *in vivo*.
4. A significant difference was found between Vitremer and Right-On ($p=0.033$), but the difference between Dyract and Right-On was not significant ($p=0.179$).
5. The difference between Vitremer and Dyract ($p=0.318$) and Right-On and Right-On ($p=0.899$) were not statistically significant.
6. The fluoride-releasing materials, Vitremer and Dyract, were found to differ significantly from the non fluoride-releasing control material, Right-On, in relation to the degree of decalcification observed ($p=0.015$).

3.2.2 MINERAL PROFILES OF WHITE SPOT LESIONS

Microradiography

Radiographs of 94 sections of premolars were examined under a light microscope (Carl, Zeiss) for early caries lesions (magnification=10X). Two teeth, that had scored 4 (frank cavitation), were not analysed by microradiography.

A summary of the findings is as follows:

1. Caries was found on 11 sections (12%). In 9 sections, brackets had been bonded with Right-On (10%), one with Dyract (1%), and the remaining one with Vitremer (1%). This result is similar to that found by indirect visual assessment (Table 3.7).
2. Three more minimal caries lesions were detected on the micro-radiographs which were not evident on visual examination.
3. Overall, 6% of the test teeth (3 out of 48 in total) and 21% of the control teeth (10 out of 48 in total) demonstrated caries lesions.

Microdensitometry

All eleven sections that had shown caries lesions were subjected to further microdensitometric analysis. Mineral profiles of these lesions were quantified and the measured parameters of the total mineral loss (Δz), lesion depth (Ld), surface zone mineral content (SZ) and lesion body mineral (LB) content calculated (Table 3.8).

The results were as follows:

1. All teeth that scored 3 by indirect visual assessment were confirmed to have surface demineralisation by means of microdensitometry.
2. Among the lowest in lesion depth (Ld) and integrated mineral loss (Δz) were the three lesions (1=Vitremer, 1=Dyract, 1=Right-On) which did not show white spots on visual examination.
3. Out of the three test teeth (bonded with fluoride-releasing cements) which had confirmation of demineralisation, one was cavitated and had been bonded with Dyract, one was bonded with Vitremer (Ld=51.74μm), and the remaining one was bonded with Dyract (Ld=112.75μm).

Table 3.7

Early caries lesions (white spot lesions) detected by indirect visual assessment and /or microradiography

	Microradiography		
Indirect visual assessment (score 3 or 4)	Yes	No	Total
Yes	10	0	10
No	3	83	86
Total	13	83	96

Table 3.8

Mineral profile of premolars, with white spot lesions (n=11), that scored 3 (modified von der Fehr Index) by indirect visual assessment

	Lesion depth Ld (um)	Integrated mineral loss Δ z (%vol um)	Surface zone mineral content SZ (%vol)	Lesion body mineral content LB (%vol)
median	93.6	1215	61.8	57.2
Q1, Q3	72.3 112.5	713 1599	0.00 69.69	21.27 62.36
min, max	45.2 359.7	531 4535	0.00 83.42	18.34 73.70

1. There was great variation in lesion depth (Ld) and integrated mineral loss (Δz) in sections which had scored 3 by indirect visual assessment (Fig. 3.1, 3.2 and 3.3).

   With such a small number of test teeth (1 with Vitremer, 2 with Dyract) shown to have demineralisation, no formal statistical analysis was carried out to compare the mineral profiles of these lesions with those of the control teeth with caries.

3.2.3 SALIVARY FLUORIDE

Saliva samples were collected on two occasions, pre-bonding and 4 weeks post-bonding. There were 52 salivary samples altogether; 26 were from patients bonded with Vitremer and the remaining 26 from subjects bonded with Dyract. Fluoride ion concentration in saliva collected on the two occasions are tabulated in Table3.9.

'Boxplots' in Figure 3.4 and Figure 3.5 show that the data were distributed asymmetrically. The sample median is a more 'representative' value than the sample mean under such circumstances. As the data were not distributed normally, the natural logarithm of the data was taken to proceed with further analysis. The median of the difference in salivary fluoride ion concentration between visit 2 and visit 3 are tabulated in Table 3.10. t-tests were carried out to assess whether there was any significant difference between the two occasions.

The findings were as follows:

The difference in salivary fluoride ion concentration between visit 2 and visit 3 was very small in both groups (Table 3.9). Both the pre-bonding and post-bonding salivary fluoride ion concentrations were below the normal

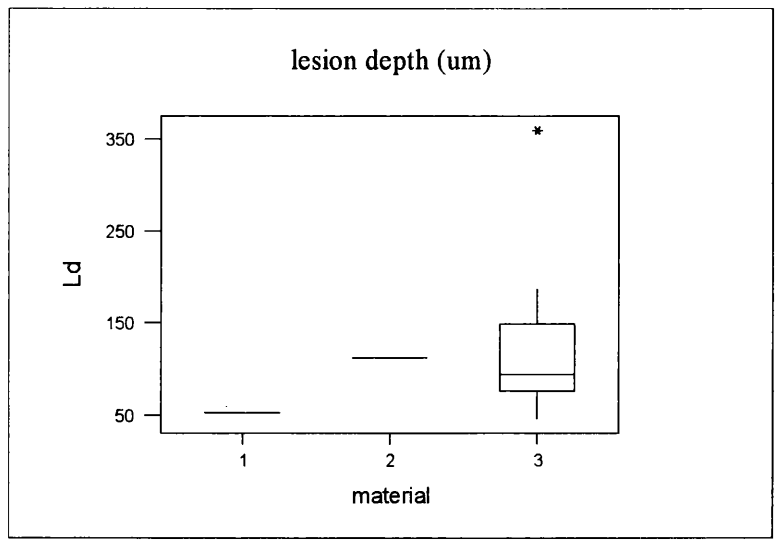


Fig. 3.1 Lesion depth (um) of caries lesions in premolars bonded with Vitremer (1), Dyract (2) and Right-On (3)

material 1= Vitremer
2= Dyract
3= Right-On

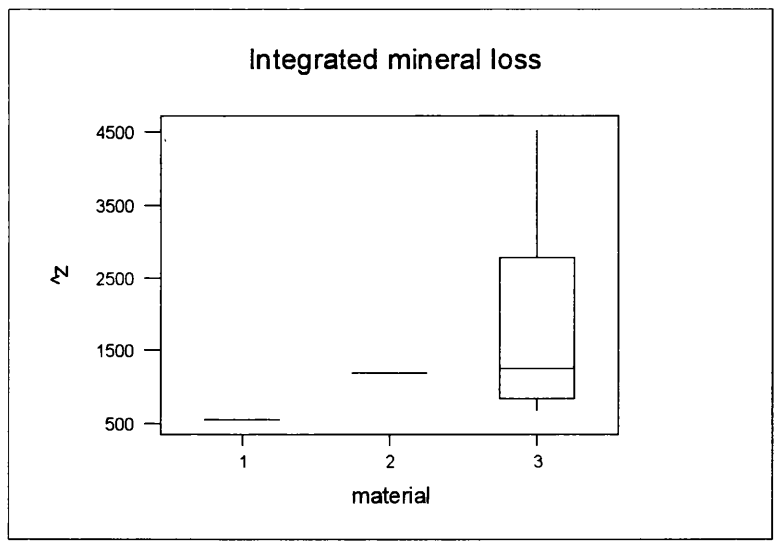
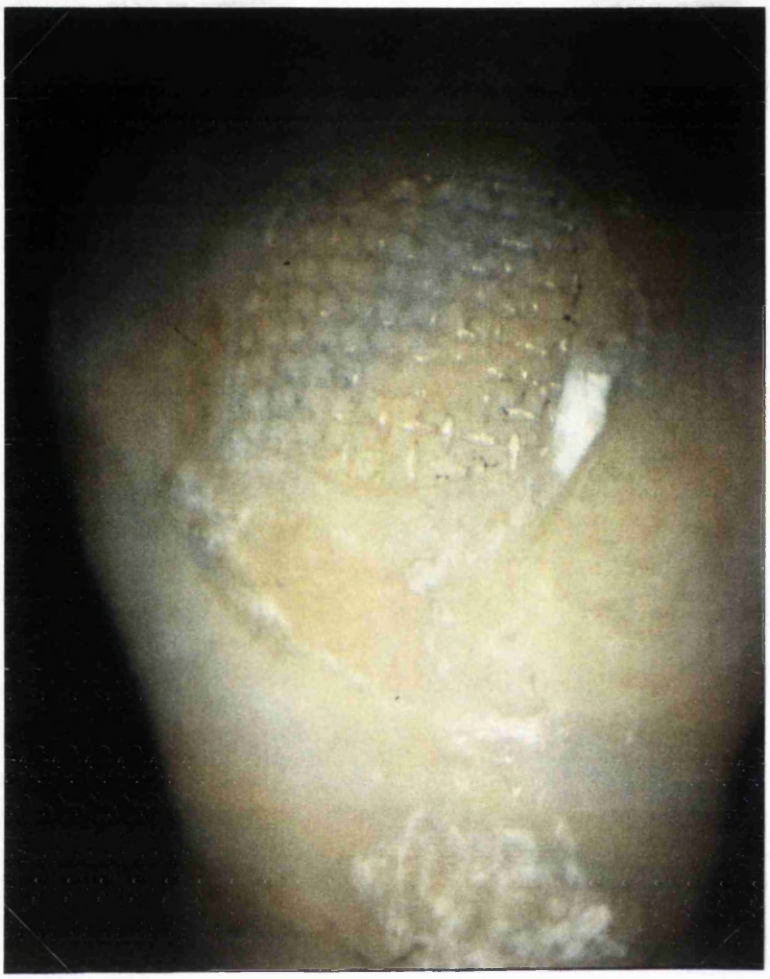


Fig. 3.2 Integrated mineral loss (%vol.min um) in caries lesions in premolars bonded with Vitremer (1), Dyract (2), and Right-On(3)

material 1= Vitremer
2= Dyract
3= Right-On



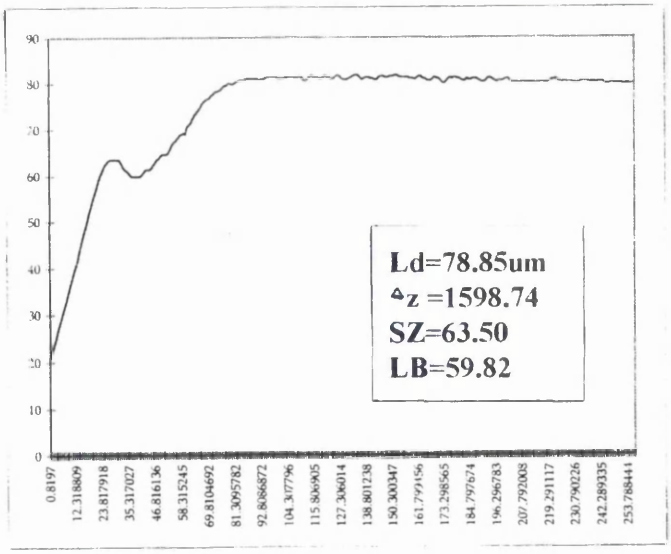
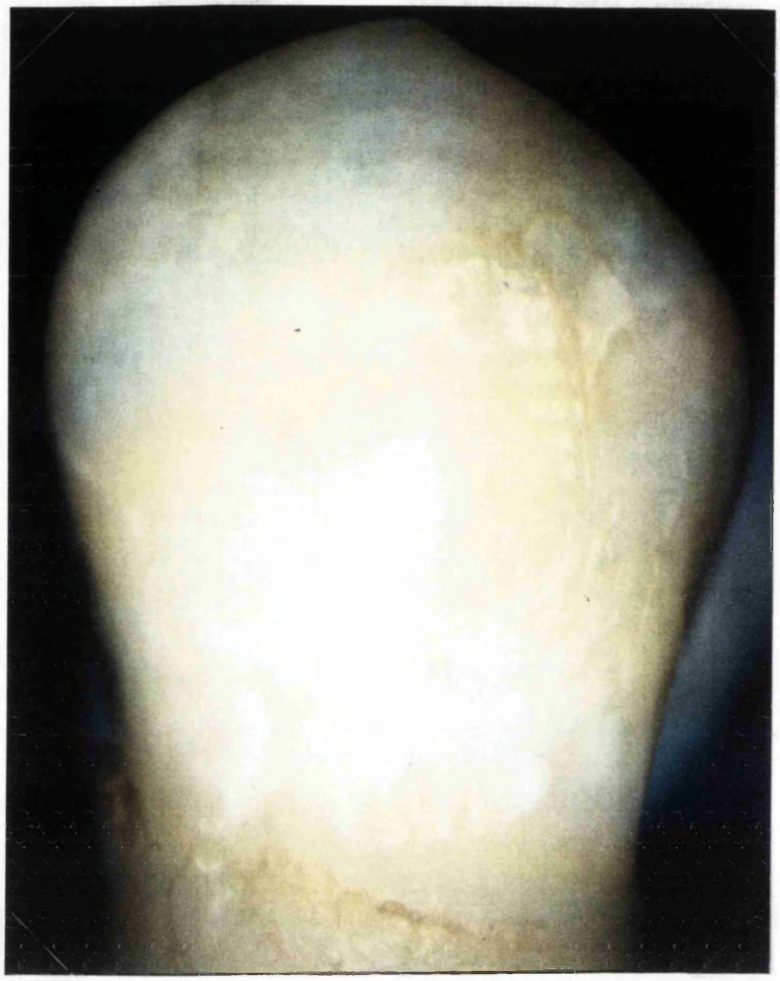


Fig. 3.3.1 (Top) colour print of a tooth (bonded with Right-On) that scored 3 [visual assessment]
(Bottom) mineral profile [microdesitometric analysis] of the most severe lesion selected from the tooth shown above



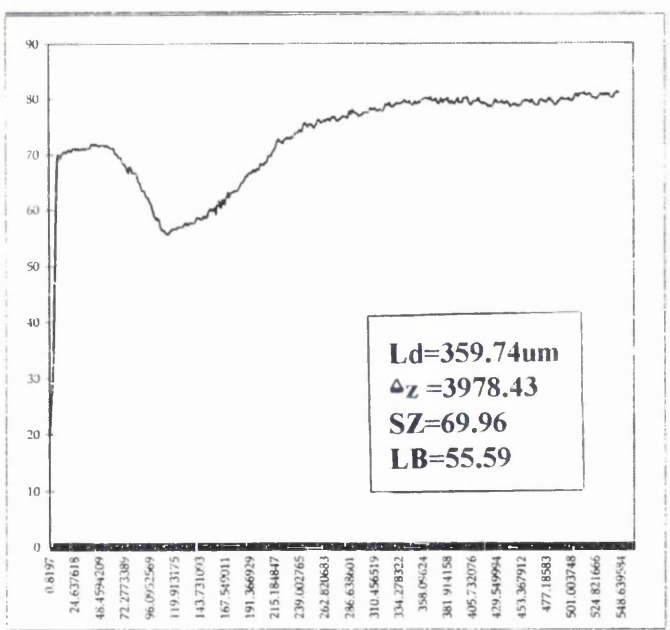
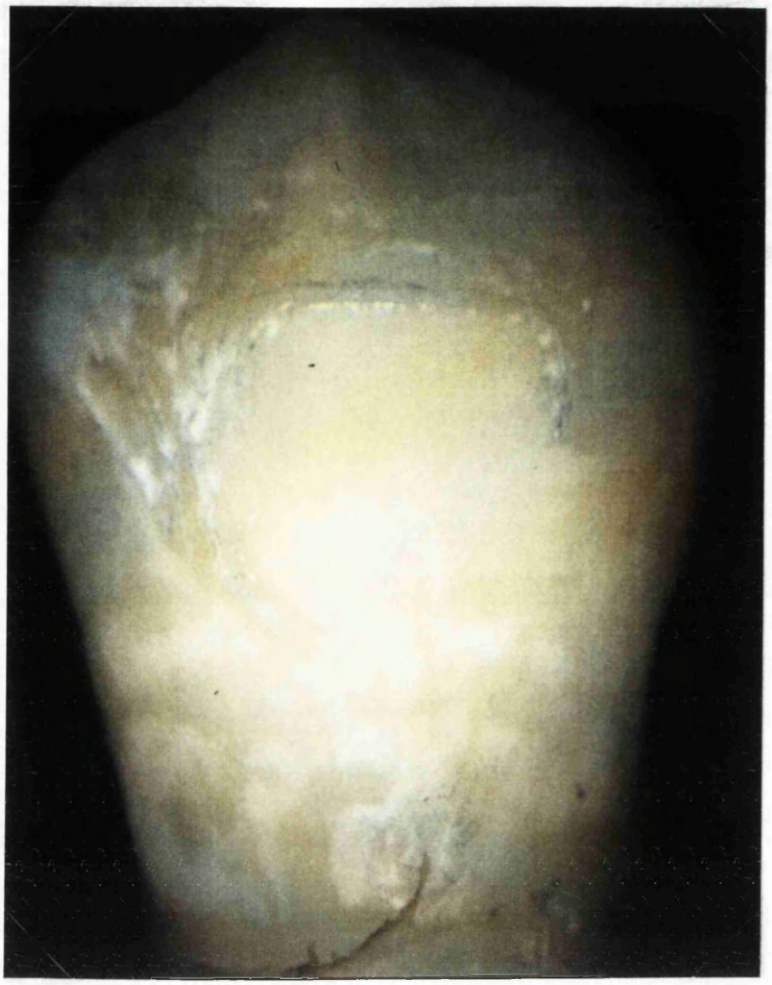


Fig. 3.3.2 (Top) colour print of a tooth that scored 3 (bonded with Right-On) [visual assessment]
(Bottom) mineral profile [microdesitometric analysis] of the most severe lesion selected from the tooth shown above



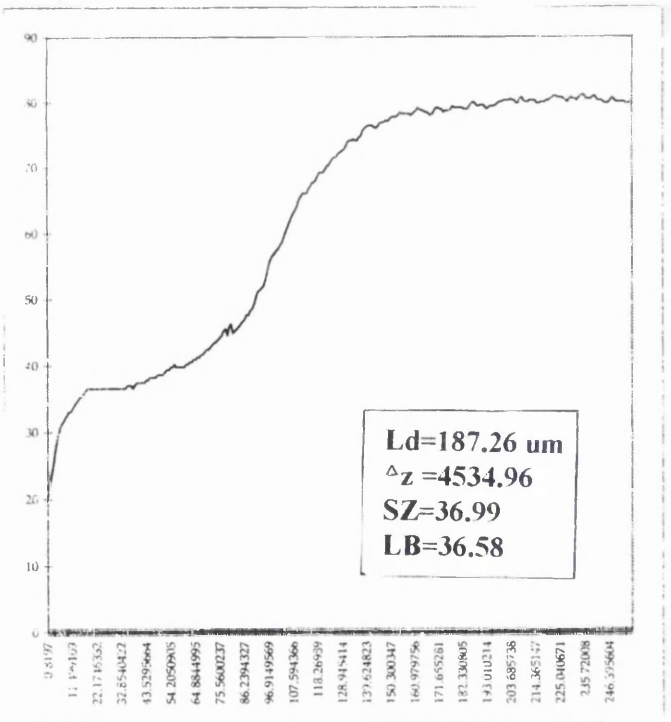


Fig. 3.3.3 (Top) colour print of a tooth (bonded with Dyract) that scored 3 [visual assessment]
(Bottom) mineral profile [microdesitometric analysis] of the most severe lesion selected from the tooth shown above

Table 3.9
Salivary fluoride ion concentration on Visit 2 (pre-bonding) and Visit 3 (4 weeks post-bonding) for subjects who had orthodontic brackets bonded with the test materials.

	Material			
	Vitremer		Dyract	
	Pre-bonding [F-] ppm	Post-bonding [F-] ppm	Pre-bonding [F-] ppm	Post-bonding [F-] ppm
Number of subjects	13	13	13	13
Median	0.011	0.012	0.005	0.009
Interquartile range (Q1-Q3)	0.008-0.030	0.008-0.040	0.004-0.010	0.004-0.025
Min, Max	0.006, 0.062	0.004, 0.107	0.003, 0.020	0.003, 0.095

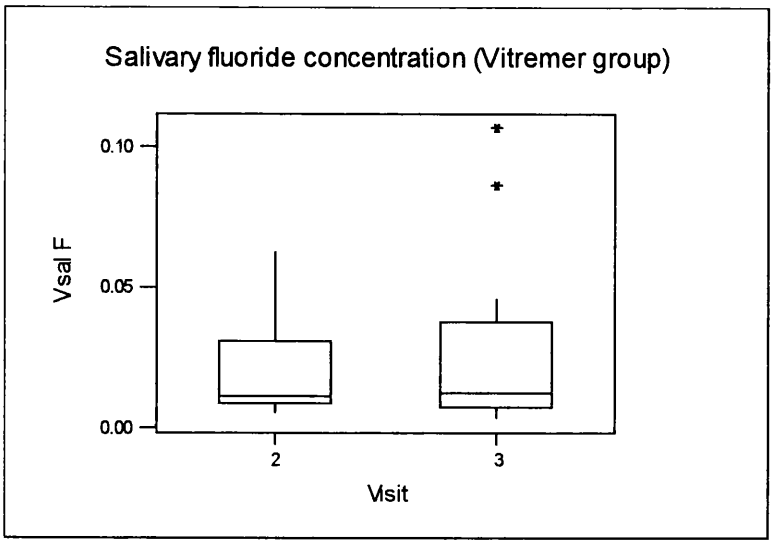


Fig. 3.4 Salivary fluoride concentration (visit 2 and visit 3) in subjects whose premolars had been bonded with Vitremer as test material.

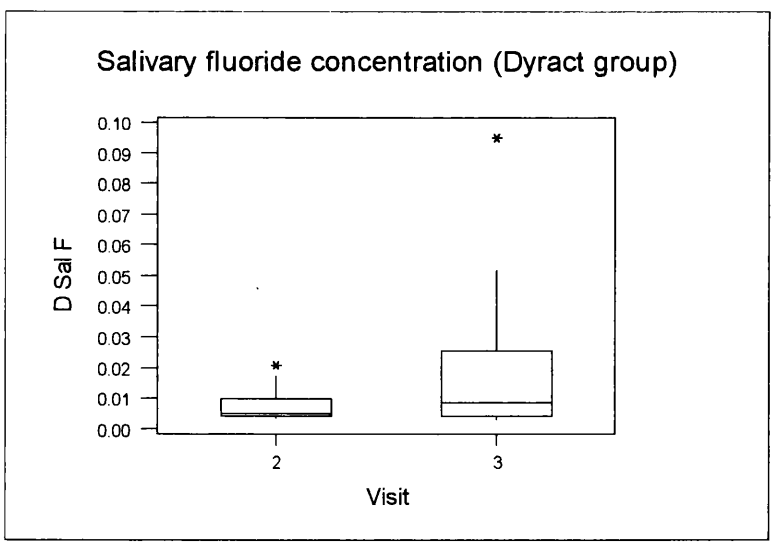


Fig. 3.5 Salivary fluoride concentration (visit 2 and visit 3) in subjects whose premolars had been bonded with Dyract as test material.

Table 3.10
Median and results of two sample t-tests (p- value) of the difference in salivary fluoride ion concentration between visit 2 (pre-bonding) and visit 3 (4 weeks post-bonding).

	Material	
	Vitremer	Dyract
Number of subjects	13	13
Median	-0.004	0.001
Interquartile range (Q1-Q3)	-0.018 - 0.014	-0.001 - 0.020
P - value (95% C.I.)	0.72	0.14

background level range of 0.02 to 0.04 ppm for people living in an area where drinking water is not fluoridated.

2. Neither Vitremer nor Dyract had altered the salivary fluoride ion concentration significantly 4 weeks post bonding (Table 3.10). The difference in salivary fluoride ion concentration between visit 2 and visit 3 was not statistically significant in subjects who had brackets bonded with Vitremer ($p = 0.72$) or Dyract ($p = 0.14$).

3.2.4 PLAQUE FLUORIDE

Plaque samples were collected from each premolar tooth involved in the study on Visit 2 and Visit 3. 192 plaque samples were collected on the two occasions. The concentration of fluoride in plaque (ppm/mg wet weight) is tabulated in Appendix XI.

The data were not normally distributed, and a sample median and interquartile range were used to describe the spread of the data instead of a sample mean and standard deviation. Table 3.11 and Table 3.12 show the sample median and interquartile range for teeth bonded with Vitremer and Dyract .

To find out whether the difference in fluoride concentration was statistically significant between Visit 2 and Visit 3, for each material all the data were normalized by taking the natural logarithm. t-tests were then performed, and the results are tabulated in Table 3.13.

t-tests were also carried out to compare the performance (fluoride releasing ability) of the test and control materials. Results are shown in Table 3.14.

Table 3.11
Fluoride ion concentration in plaque (ppm/mg wet weight) in subjects who had premolar teeth bonded with Vitremer (test material) and control teeth bonded with Right-On (control material) on Visit 2 (pre-bonding) and Visit 3 (4 weeks post bonding).

Q1 - Q3 = Interquartile range

	Visit 2 (pre- bonding)			
	Upper		Lower	
Material	Test	Control	Test	Control
	Vitremer	Right-On	Vitremer	Right-On
Number	13	13	12	12
Median	0.85	0.70	0.70	1.36
Q1 - Q3	0.33- 3.67	0.24- 1.49	0.24- 1.36	0.24- 4.79
	Visit 3 (4 weeks post- bonding)			
	Upper		Lower	
Material	Test	Control	Test	Control
	Vitremer	Right-On	Vitremer	Right-On
Number	13	13	12	12
Median	2.94	0.64	3.64	1.32
Q1 - Q3	0.72- 7.91	0.30- 2.85	0.85- 6.64	0.28- 4.65

Table 3.12
Fluoride ion concentration in plaque (ppm/mg wet weight) in subjects who had premolar teeth bonded with Dyract (test material) and control teeth bonded with Right-On (control material) on Visit 2 (pre-bonding) and Vist 3 (4 weeks post bonding).

Q1 - Q3 = Interquartile range

	Visit 2 (pre- bonding)			
	Upper		Lower	
Material	Test	Control	Test	Control
	Dyract	Right-On	Dyract	Right-On
Number	12	12	11	11
Median	1.60	2.4	0.57	0.92
Q1 - Q3	0.75- 2.63	0.60- 4.70	0.22- 4.64	0.67- 1.31
	Visit 3 (4 weeks post- bonding)			
	Upper		Lower	
Material	Test	Control	Test	Control
	Dyract	Right-On	Dyract	Right-On
Number	12	12	11	11
Median	2.37	0.90	2.62	0.69
Q1 - Q3	0.25- 3.48	0.34- 4.57	0.31- 6.54	0.28- 3.67

Table 3.13
Difference in fluoride ion concentration for premolar teeth bonded with one of the two test materials between Visit 2 and Visit 3 . All the data were normalized by taking the natural logarithm. t-tests and confidence interval at 95% were performed.

Difference in fluoride ion concentration in plaque for:
V2- V1= premolar teeth bonded with Vitremer in visit 3 and visit 2 respectively
D2- D1= premolar teeth bonded with Dyract in visit 3 and visit 2 respectively
R2- R1= premolar teeth bonded with Right-On in visit 3 and visit 2 respectively

	Upper arch			
	Vitremer	Right-On	Dyract	Right-On
	Test	Control	Test	Control
	log (V2- V1)	log (R2- R1)	log(D2- D1)	log(R2- R1)
Number	13	13	12	12
Median	0.717	0.201	-0.001	-0.563
Q1 - Q3	-0.223 - 1.880	-0.970 - 1.233	-2.005 - 0.841	-2.367 - 0.468
95% C.I.	(0.083, 1.531)	(-1.091, 1.055)	(-2.020,1.743)	(-1.856, 0.432)
P-value	0.032	0.97	0.87	0.20
Lower arch				
	Vitremer	Right-On	Dyract	Right-On
	Test	Control	Test	Control
	log (V2- V1)	log (R1- R2)	log (D2 - D1)	log (R2- R1)
Number	12	12	11	11
Median	1.267	0.301	0.772	0.383
Q1 - Q3	0.223 - 2.942	-0.895 - 1.745	-0.952 - 2.236	-1.900 - 1.733
95% C.I.	(0.400, 2.596)	(-1.162, 1.413)	(-0.513, 2.109)	(-1.548, 1.335)
P- value	0.012	0.83	0.20	0.87

Table 3.14

Comparision of change in fluoride ion concentration in plaque (visit 2 v visit 3) between different groups bonded with test and control materials.The natural logarithm was taken to normalise the data before confidence interval and p-values were determined by t-tests.

V= Vitremer
D= Dyract
R= Right-On

	Upper arch		
	log (difference in [F-] in plaque between visit 2 and 3)		
	V v D	V v R	D v R
95% C.I.	(-1.02, 2.91)	(-0.41,2.06)	(-1.53,2.68)
P -Value	0.32	0.18	0.57
	Lower arch		
	log (difference in [F-] in plaque between visit 2 and 3)		
	V v D	V v R	D v R
95% C.I.	(-0.91,2.31)	(-0.23,2.97)	(-0.93,2.73)
P - Value	0.37	0.09	0.31
	Upper and lower arch		
	log (difference in [F-] in plaque between visit 2 and 3)		
	(V + D) v (R + R)		
95% C.I.	(-0.04, 3.73)		
P - Value	0.05		

A summary of the findings are as follows:

1. Premolars bonded with Vitremer showed an increase in fluoride ion concentration in plaque both in the upper and lower arch. The sample median increased from 0.85 to 2.94 in the upper arch and changed from 0.70 to 3.64 in the lower arch.
2. Premolars bonded with Dyract also showed an increase in fluoride concentration in plaque both in the upper and lower arch.The sample median increased from 1.60 to 2.37 in the upper arch while in the lower arch it changed from 0.57 to 2.62.
3. The increase in fluoride concentration in plaque was greater in the group bonded with Vitremer.
4. Premolars bonded with the control material, Right -On, did not show any increase in fluoride ion concentration in plaque.
5. There was a significant increase in fluoride ion concentration in plaque around the brackets bonded with Vitremer both in the upper arch ($p=0.032$) and in the lower arch ($p=0.012$) 4 weeks after the brackets were in place.
6. The fluoride ion concentration in plaque around brackets bonded with Dyract or Right-On did not show any significant increase between visit 2 and visit 3.
7. There was no significant difference in plaque fluoride concentration between the two test materials in the upper arch ($p=0.32$) or lower arch ($p=0.37$).
8. There was no significant difference in plaque fluoride concentration between Vitremer and Right-On or between Dyract and Right-On.
9. The difference in plaque fluoride concentration between the fluoride-releasing materials, as a group, and the control material was not statistically significant ($p=0.05$).

3.2.5 URINARY FLUORIDE

Urine samples were collected on two occasions, pre-bonding and 4 weeks post-bonding. There were 52 urine samples in total, 26 were from patients bonded with Vitremer and 26 from patients bonded with Dyract. Table 3.15 shows the mean, standard deviation, median and interquartile range of fluoride ion concentration in urine collected on the two occasions.
As illustrated in the 'Boxplots', Figure 3.6 and Figure 3.7, there were one or two "outliers" which may have distored the mean values and the sample medians were regarded as more meaningful under such circumstances.

The difference in fluoride ion concentration (ppm) between visit 2 and visit 3 are tabulated in Table 3.16. t-tests were then carried out to assess whether there was any difference between these two occasions.

A summary of the findings is as follows:

1. After the 4 week 'wash out' period, using a non-fluoride toothpaste instead of a normal household brand, the medians of urinary fluoride ion concentration in subjects bonded with Vitremer and Dyract were 0.416ppm and 0.383ppm respectively.
2. Four weeks after brackets were bonded with the test materials, the medians of urinary fluoride ion concentration in subjects bonded with Vitremer and Dyract were 0.387ppm and 0.396 ppm respectively.
3. The sample medians of the *difference* in fluoride concentration were 0.051 ppm and 0.048 ppm for the Vitremer and Dyract groups respectively.

Table 3.15
Urinary fluoride ion concentration on Visit 2 (pre-bonding) and Visit 3 (4 weeks post-bonding) for subjects who had orthodontic brackets bonded with the test materials.

	Material			
	Vitremer		Dyract	
	Pre-bonding [F-] ppm	Post-bonding [F-] ppm	Pre-bonding [F-] ppm	Post-bonding [F-] ppm
Number of subjects	13	13	13	13
Mean	0.522	0.434	0.391	0.453
Standard deviation	0.400	0.195	0.186	0.216
Median	0.416	0.387	0.383	0.396
Interquartile range (Q1-Q3)	0.269-0.620	0.271-0.638	0.237-0.606	0.277-0.619

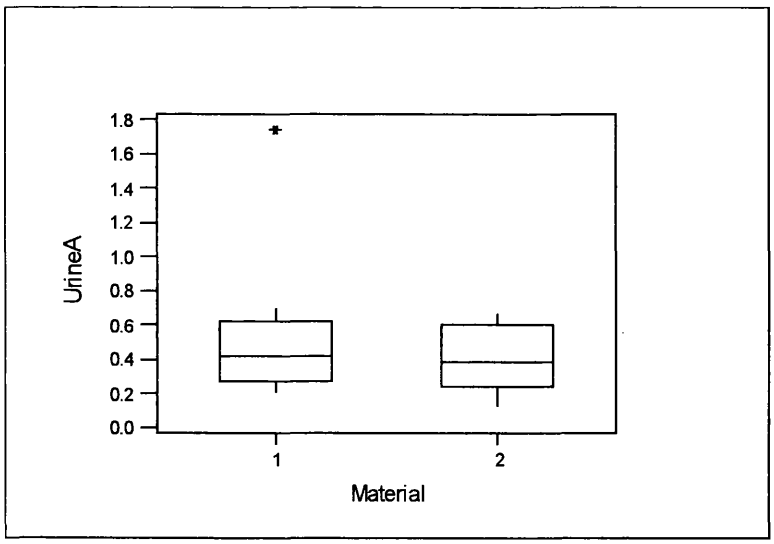


Figure 3.6
Urinary fluoride concentration before brackets were bonded with test materials.

UrineA=Fluoride ion concentration(ppm) pre-bonding
1= Vitremer 2=Dyract

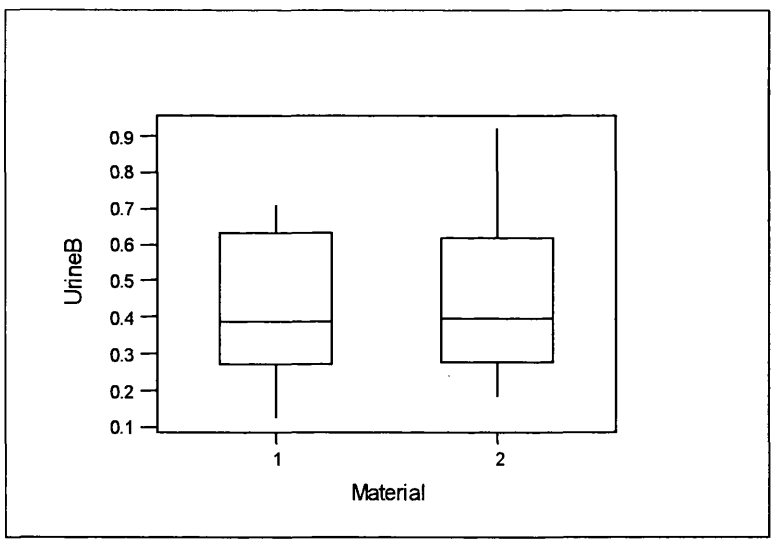


Figure 3.7
Urinary fluoride concentration 4 weeks after brackets were bonded with test materials.

UrineB=Fluoride ion concentration(ppm) post-bonding
1= Vitremer 2=Dyract

Table 3.16
Difference in urinary fluoride ion concentration between visit 2 (pre-bonding) and visit 3 (4 weeks post-bonding)

	Material	
	Vitremer	Dyract
Number of subjects	13	13
Mean	-0.088	0.0616
Standard deviation	0.357	0.157
Median	0.051	0.048
Interquartile range (Q1-Q3)	-0.165 - 0.131	-0.004 - 0.208
95% C.I.	-0.17, 0.348	-0.225,0.102
p-value	0.49	0.44

1. There was no significant difference between the two test groups in urinary fluoride concentration between visit 2 and visit 3 (95%C.I. -0.379, 0.080 ; p=0.19)
2. There was no statistical difference in urinary fluoride concentration in subjects who had brackets bonded with Vitremer (95% C.I. -0.17, 0.348; p =0.49). The difference was also insignificant in the group which had brackets bonded with Dyract (95% C.I.-0.225, 0.102; p =0.44).

CHAPTER 4

DISCUSSION AND CONCLUSIONS

The presence of white-spot lesions around orthodontic brackets represents a serious problem of clinical importance, even five years after treatment has been completed (Ogaard, 1989). Recent *in vivo* studies have shown that glass ionomers may inhibit lesion development when used to retain orthodontic brackets (Marcusson *et al.*, 1993, 1995). This is generally attributed to the fluoride that is released from the cement.

Resin-modified glass ionomer cements combine the desirable characteristics of glass ionomer cement and composite resin, and have been shown to release a significant amount of fluoride *in vitro* (Creanor *et al.*, 1994; Chadwick and Gordon, 1995). The study reported here, was undertaken *in vivo* to compare the cariostatic ability of two resin-modified glass ionomer cements and to assess the release of fluoride into saliva, plaque and urine from these two cements compared to that from a control material.

4.1 *VALIDITY OF STUDY DESIGN AND ASSESSMENT METHODS*

- To isolate specifically the effect of fluoride released from the resin-modified glass ionomer cements, several measures were adopted as discussed in section 2.2. A split-mouth study design was used in this investigation, as this has been shown to minimize any possible crossover effects of fluoride ion concentration (Hallgren *et al.*, 1992, 1993).
- The method used to analyse fluoride ion concentration in this study has been proven to be reliable and reproducible (Creanor, 1987).

- To facilitate objective assessment of decalcification, an indirect visual assessment using colour transparencies was used in this study. The rationale for choosing this indirect method was as follows:

1. It allowed an objective assessment of decalcification to be made.
2. It was possible to repeat the assessment without recourse to the patient.
3. Intra- and inter-examiner reliability could be assessed relatively easily.

Despite the obvious advantages of indirect assessment, only 7 out of the 26 previous studies (less than 30%) into decalcification related to fixed orthodontic treatment used this method of assessment. Various indices have also been used to score the presence of decalcification and its extent. There is, however, no consensus on a standardised procedure to assess white spot lesions.

It was clear from the present study, that when using the modified von der Fehr index to assess decalcification, scores of 0, 3 and 4 accorded well with macroscopic (i.e. clinical) observations while scores 1 and 2 were more variable. The modified von der Fehr Index was reliable in its ability to distinguish between 'no white spot', 'a frank white spot lesion' and 'cavitation'. Further fine distinction of the degree of blemish was less reliable (scores 1 and 2), probably due to artefacts introduced through camera flash back, magnification, two dimensional view of a three dimensional object and the intensity of the background lighting. Ideally, transparency records should record the clinical picture accurately with minimal artefacts. The photographic technique employed should be standardised and aimed at producing images with the same background lighting. These conditions were met in relation to the photographic records taken in the present study. Ideally, the images recorded should not be enlarged but should be life-size images (x1) and possibly three images should be taken (right lateral, frontal and left lateral) to allow

a comprehensive assessment of decalcification to be made while hopefully reducing the incidence of artefacts.

4.2 *SIGNIFICANCE OF STUDY RESULTS*

White spot lesions

Overall, 2% of premolars bonded with the fluoride-releasing glass ionomer cements (Vitremer or Dyract) in contrast to 19% of premolars bonded with the nonfluoride-releasing material (Right-On) developed early carious lesions or cavitation around orthodontic brackets after four weeks *in vivo*. This is strong evidence that the two test fluoride-releasing resin-modified glass ionomer cements, as a group, were more effective in the prevention of white spot lesions than the control material ($p=0.015$).

These findings are similar to those of Sonis and Snell (1989), Underwood *et al.* (1989) and Marcusson *et al.* (1993, 1995), but those studies were conducted over 25 months, 60 days and 8-39 months. To date, there would appear to be no other study in which decalcification was assessed macroscopically after 1 month.

The major shortfall in all previous studies, however, has been the lack of control of the external source of fluoride ions, such as the use of fluoride toothpaste during the trial period. Furthermore, sample sizes of the study by Sonis and Snell (1989) and Underwood *et al.* (1989) were all smaller (22 patients and 10 patients respectively) than that of the present study. Besides that, only Marcusson *et al.* (1993,1995) used indirect visual assessment of white spot lesions.

More recently, Silverman *et al.* (1995) reported no decalcification on teeth bonded with a tri-cured resin-modified glass ionomer cement (Fuji Ortho LC). A larger sample size of 152 cases (3226 bonded teeth) was used, but there was no mention of the method of assessment of decalcification. It would appear to have been entirely subjective. Furthermore, it was not clear whether fluoride supplements, professional hygiene measures or fluoride toothpaste were used during the trial period.

Other previous *in vivo* studies (Mitchell, 1992; Eliades *et al.*, 1992; Millett *et al.*, 1993), however, have failed to show a significant difference between fluoride-releasing and non fluoride-releasing cements. Sample sizes of Mitchell's and Millett's were similar to the present study but the study by Eliades *et al.* (1992) included only 10 patients.

Mineral profile of lesions

The results of the microscopic examination accord well with the macroscopic findings. Fewer test teeth were affected by demineralisation and cavitation (3 in total), compared with the control teeth (10 in total).

Previous *in vivo* studies that assessed the cariostatic properties of fluoride releasing materials (composite and glass ionomer cements) four weeks post-bonding have all found a reduction in lesion depth with these materials. Rezk-Lega *et al.* (1991) with a sample of 5 patients (18 premolars), recorded a 55% and 63% reduction in lesion depth with Ketac-Cem and Aqua-Cem respectively whilst Ogaard (1992) with a similar sample size (6 patients-20 premolars), found a 48% reduction in lesion depth with a fluoride-releasing resin (VP 862).

The median lesion depth ($Ld = 93.6 \mu m$) and integrated mineral loss ($\Delta z = 1250$) for premolars, which were bonded with non-fluoride-releasing

materials, in the present study parallels a similar study by Ogaard *et al.* (1988). Out of their 5 patient sample (10 premolars), four weeks post-bonding, the mean Ld and Δz were 101 +/- 26 μm and 1525+/- 460 respectively. When these results were compared with another *in vivo* study (Ogaard *et al.*, 1992), where fluoride toothpaste was used during the trial period, Ld was found to be less (61+/- 12μm). This highlighted that the use of fluoride toothpaste during a clinical trial reduces lesion depth and enhances the cariostatic potential of any fluoride-releasing material. The present study used a larger sample size (48 test and 48 control teeth). Of these, 10 control teeth developed frank white spots or cavitation while 3 test teeth developed similar lesions.

In the present study premolars that scored 3 in the indirect visual examination, showed great variation in their mineral profile. The integrated mineral loss (Δz) varied from 5204 to 531 %vol.min μm and lesion depth (Ld) ranged from 45.2 to 424.6μm, which indicated a ten fold difference in both parameters. This can be explained by a variety of reasons:

1. A higher and more or less continuous cariogenic challenge in association with fixed orthodontic treatment in certain individuals.
2. Different rates and patterns of demineralisation and remineralisation were reflected by the presence of various types of lesions, such as surface softening defects, subsurface lesions, and laminated lesions. With all the patients adhering to the same protocol, it seems that caries susceptibility differed greatly between patients.

Previous laboratory and clinical studies (Swartz *et al.*, 1984; Hallgren *et al.*, 1990; Ghani *et al.*, 1994) have shown that the fluoride released from glass ionomer cement is greatest during the first 24 hours post bonding.

The protective effect of fluoride against demineralisation observed in the present study could be accounted for by various factors:

- It may be that a relatively high concentration of fluoride ions diffused onto the enamel surface during the initial 'burst-effect' of ionic fluoride release, increasing both the hardness and acid resistance of the enamel to subsequent demineralisation (Creanor *et al.*, 1994).
- On the other hand, formation of calcium fluoride on the enamel surface may act as a potent reservoir of fluoride, slowly releasing ions available for use in remineralisation or redeposition into areas of demineralisation (Arends *et al.*, 1984).

The clinical significance of the present study is that enamel demineralisation around orthodontic brackets is a very rapid process in the absence of fluoride supplements. Visible white spots lesions and cavitation developed within four weeks - that is, within the interval from one orthodontic treatment appointment to the next.

All the eight teeth that had frank white spot lesions were bonded with the non fluoride-releasing cement. Cavities were found in two teeth after only 4 weeks. This degree of decalcification has not been previously reported elsewhere. The fact that both cavities were found in the same mouth, however, indicates one individual's increased susceptibility to caries.

Salivary fluoride

Neither Vitremer nor Dyract altered the salivary fluoride ion concentration significantly at four weeks post bonding. The difference in salivary fluoride ion concentration between visit 2 and visit 3 was very small in both groups. The baseline mean fluoride ion concentration of whole saliva in this study (0.014 ppm) was similar to that in Hallgren's (1990) study (0.016ppm) using Ketac Cem as the bonding agent. Although Hallgren *et al.* (1990)

found a significant increase in salivary fluoride concentration *one* day after bracket cementation, the salivary fluoride concentration after 28 days was similar to that found in the present study. In the study reported here the salivary fluoride ion concentration was found to be slightly elevated from the baseline level but the increase was not significant.

Plaque fluoride

The concentration of fluoride in plaque was evaluated at both visit 2 (pre-bonding) and visit 3 (post-bonding). Plaque fluoride concentration around orthodontic brackets bonded with Vitremer or Dyract was increased after four weeks. This occurred in both upper and lower arches. By contrast, Right-On (control material) demonstrated no increase in plaque fluoride concentration. The difference in plaque fluoride concentration between the fluoride-releasing and non fluoride-releasing materials was not statistically significant ($p=0.05$).

Hallgren (1993) found the plaque fluoride concentration elevated significantly after 6 months when using glass ionomer cement (Ketac-Cem) as a bonding agent. In the present study, a smaller increase in plaque fluoride concentration was observed compared to that found by Hallgren (1993). The apparent disparity in plaque fluoride concentration between these studies can be explained by various factors:

- The design of the present study minimized the possibility that the fluoride released from the cements was replenished by fluoride from principle extrinsic sources. i.e. the drinking water and fluoride toothpaste. The use of a non-fluoride toothpaste throughout the trial period was only adopted in this and one other study (Rezk-Lega *et al.*, 1991).
- Before plaque samples were collected, patients had been asked to refrain from oral hygiene measures for 24 hours in this study instead of for 48

hours as in Hallgren's (1993) study. Consequently the thickness of plaque present may have been different and therefore, may have influenced the fluoride concentration.

Low level fluoride in the fluid phase surrounding the teeth is known to have substantial cariostatic potential by inhibiting demineralisation and enhancing remineralisation (Brunn and Thylstrup, 1984). The results of the present study suggest that release of fluoride from compomer cements increases the plaque fluoride concentration, thereby enhancing the local cariostatic effect.

Urinary fluoride

Fluoride released from any fluoride-releasing cement is subsequently distributed into body fluids, including saliva and gingival fluid, after it is swallowed. Urinary fluoride ion concentration is widely regarded as a reliable indicator of fluoride intake. When the water fluoride level is not more than 0.2 ppm, the urinary fluoride concentration reflects intake from external sources.

To date, there has been no report on the systemic uptake of fluoride from fluoride-releasing cements. In the present study, the difference in urinary fluoride ion concentration between visit 2 and visit 3 was remarkably small in both test groups. Neither Vitremer nor Dyract altered the urinary fluoride ion concentration significantly 4 weeks post bonding. This indicated that the cariostatic effect of the fluoride releasing materials is essentially a local one; the contribution through systemic fluoride uptake is, therefore, negligible. It also confirmed that the resin-modified glass ionomer cements used in the present study are not contraindicated on toxicological grounds.

4.3 *CONCLUSIONS AND SUGGESTIONS FOR FURTHER WORK*

CONCLUSIONS:

The aims of this thesis were outlined in section 1.1. The conclusions relating to each are given here:

Aim 1. To compare the cariostatic ability of two resin-modified glass ionomer cements (Vitremer and Dyract) to that of a conventional adhesive resin (Right-On).

Conclusion:

The cariostatic properties of the fluoride-releasing resin-modified glass ionomer cements were significantly better than the control material Right-On ($p=0.015$). Vitremer appeared to be slightly more effective in preventing white spot lesions than Dyract, but the evidence was inconclusive.

Aim 2. To compare the local and systemic uptake of fluoride from two resin-modified glass ionomer cements (Vitremer and Dyract) with those from a conventional adhesive resin (Right-On) by quantifying the fluoride concentration in plaque, saliva, and urine.

Conclusion:

Both Vitremer and Dyract increased plaque fluoride concentration, while Right-On did not. There was no convincing evidence of any systemic uptake of fluoride from Vitremer or Dyract when the fluoride concentrations in urine and saliva were analysed. The cariostatic effect of these fluoride-releasing cements is therefore likely to be entirely local and not due to a systemic fluoride level.

This thesis addressed the following null hypotheses as outlined in section 1.1

1. There is no significant difference in decalcification observed with the resin-modified glass ionomer cements (Vitremer or Dyract) compared to that observed with the conventional adhesive resin (Right-On).

This null hypothesis is rejected as the results of this study showed that significantly less decalcification was observed with the resin-modified glass ionomer cements (Vitremer and Dyract) compared to that observed with the conventional adhesive resin (Right-On).

2. There is no significant difference in the local and systemic uptake of fluoride from the two resin-modified glass ionomer cements (Vitremer and Dyract) when compared with those from the conventional adhesive resin (Right-On) .

On the basis of the results of this study this null hypothesis is partly rejected as there was a significant difference in the local uptake of fluoride in plaque when comparing Vitremer with Right-On.

SUGGESTIONS FOR FURTHER WORK

From the results of this study, it appears that orthodontic patients are likely to benefit from the local cariostatic effect of resin-modified glass ionomer cements. *In vitro* studies have demonstrated the ability of resin-modified cements to release and take up fluoride. The present study confirmed their fluoride-releasing ability and documented this clinical impact four weeks post bonding. The ability to absorb fluoride ions *in vivo* from external fluoride supplements, however, remains to be explored. It is suggested, therefore that the following studies should be undertaken:

1. An *in vivo* study with regular professional cleaning of two predetermined quadrants (split-mouth technique) using normal fluoride toothpaste twice per week. Conclusions drawn from such intra-individual comparisons would throw some light as to the uptake and release characteristics of resin-modified glass ionomer cements *in vivo.*

2. Long term *in vivo* studies of fluoride-releasing resin-modified glass ionomer cements without withholding routine use of fluoride toothpaste.

3. Concerning methodology, there is a need to establish a standardised way to assess demineralisation *in vivo.* Photographic imaging for indirect assessment should mimic accurately the clinical situation regarding: size, background lighting and angulation.

4. This study has permitted a reasonable estimate of the sample size required for further decalcification studies. If an indirect assessment of decalcification is to be undertaken, using a half-mouth study design and bonding 8 brackets with a test material and 8 brackets with a control material

(assuming first premolars to be extracted), then 50 patients are likely to be required to allow an objective assessment of decalcification to be made.

APPENDIX I ETHICAL APPROVAL

Dr S L Creanor
Dept. of Oral Sciences
Glasgow Dental Hospital

Dear Dr Creanor

Area Dental Ethics Committee

Protocol: "Study of Fluoride-release and uptake from glass ionomer cements used for orthodontic purposes - an in vivo investigation."

I write to inform you that your protocol for a clinical research project has now been approved by the Area Dental Ethics Committee subject to the standard consent form being used.

The Committee would be grateful if you would inform them of the results of your project and any ethical problems encountered when the project is complete.

Yours sincerely

H A Critchlow
Chairman
Area Dental Ethics Committee

GREATER GLASGOW HEALTH BOARD

APPENDIX II EXPLANATORY NOTE

SUMMARY OF THE CLINICAL STUDY

1. Objectives of the study and guidelines for participating in the study are explained. When both the subject and parent are happy with the arrangement, a consent form is signed. A non-fluoride tooth paste is given to each subject four weeks before the brace is placed.

2. In the visit allocated to fit the brace, samples of urine, saliva and plaque are collected after 24 hrs without oral hygiene measures. Brackets are placed on teeth that are to be extracted as part of the treatment. Patients are advised to brush thoroughly twice daily as they usually do with the toothpaste provide.

3. Four weeks after placing the brackets, saliva, urine and plaque samples are collected again after 24 hrs without oral hygiene measures. The teeth that are planned for orthodontic extraction are removed at the same visit.

4. The patient is discharged from the study and an appointment is made for treatment as planned.

5. It is important to follow all the instructions given throughout the study. As special arrangements are made for the three visits in the study, I would be grateful if you would endeavour to keep the appointments as arranged. Please feel free to contact me if any problem arises.

APPENDIX III CONSENT FORM

CONSENT FORM FOR PROSPECTIVE CLINICAL BONDING TRIAL

The aim of this clinical study is to see if different types of glue used to attach the fixed brace to your teeth are capable of preventing tooth decay around the brace.

Two different types of glue are being used to attach the brace to your teeth and it will be possible to discover if one glue works better than the other in time. In addition both of the glues contain fluoride that may help prevent tooth decay starting around the fixed brace during treatment. All materials used in this study are already in everyday use in the mouth mainly for fillings.

To ensure that the only source of fluoride is from the glue, a non-fluoride toothpaste is provided to replace any normal household brand that you already use. Mouth rinses and white fillings should be avoided during the study.

Samples of plaque, saliva and urine will be taken before fitting your fixed brace and just before extractions are carried out. The teeth on which the fixed brace are placed will be extracted as part of your orthodontic treatment.

This trial will be completed within two months. It is our intention that this study does not make your treatment time any longer.

I would like to thank you for your time in reading this and would be grateful if you would consider volunteering to participate in the study. There is no cost involved and I will be happy to undertake your treatment whether or not you volunteer to take part in the clinical trial.

C. Chung
Postgraduate student in orthodontics

I hereby consent to participate in the study using two different glues to attach the fixed brace to my teeth.

Signed ---------- (parent/Guardian) Date ----------

APPENDIX IV

DATA COLLECTION FORM

STUDY NO.

HOSPITAL NO.

D.O.B.

SEX

MALOCCLUSION

START DATE **FINISH DATE**

'WASH OUT'

BONDING

MATERIAL **SITE**

	UR	UL	LL	LR
VITREMER (V)				
DYRACT (D)				
RIGHT-ON (R)				

APPENDIX V

COMPOSITION OF NON-FLUORIDE TOOTHPASTE

Abrasive	Alumina 50%
Humectant	Sorbitol 27%
Thickener	Sodium carboxymethylcellulose 0.85%
Therapeutic agent	22.15%

APPENDIX VI

Checking Electrode Slope

1. Put 50 ml distilled water and 50ml TISAB II into a 150ml beaker. Press the second function switch and the switch to record mV. Place the electrode in the solution to a depth of about 3cm.

2. After 5 minutes, pipette 1ml 100 ppm NaF standard into the solution. Stir continuously with a magnetic stirrer. Record the electrode potential in mV after 5 minutes.

3. Add 10ml 100 ppm NaF standard. Read the electrode potential in mV and record. Determine the difference between the first and second potential readings. If the change in potential is below 54mV, see the trouble-shooting check list.

APPENDIX VII

Low-level calibration curve for fluoride measurements

The following procedures are for samples containing less than $2x10^{-5}$ M or 0.4ppm fluoride and containing no fluoride complexing agents such as aluminium or iron.

1. Prepare a low-level TISAB solution by making up TISAB II without adding C.D.T.A..

2. Prepare a 10^{-3} M or 10ppm standard by diluting 0.1 M or 1000ppm standard solution. Add 50 ml low-level TISAB to 50 ml of standard.

3. To a 150ml plastic beaker, add 50ml low-level TISAB and 50 ml of deionised water. Place the electrodes in the solution. Stir thoroughly and set the meter to read mV.

4. Add increments of the TISAB to the standard solution using the steps outline in Table A.. Measure the electrode potential after each increment and plot the concentration (log axis) against the potential (linear axis). Store the final solution for checking calibration.

5. Add 50ml of low-level TISAB to 50ml of sample. Rinse the electrode, bow dry and place in the sample. Stir thoroughly. Determine the concentration of fluoride in the sample from the low-level calibration curve.

TABLE A

Additions of calibration standard with low-level TISAB to 50ml of Oxford adjustable sampler. B is a 200 - 1000 *u*l Oxford adjustable sampler. C is a 1000 - 5000 *u*l Oxford adjustable sampler.

STEP	PIPETTE	ADDED VOLUME(ml)	MOLARITY ($x\ 10^{-6}$)	PPM
1	A	0.1	1	0.01
2	A	0.1	2	0.02
3	B	0.2	4	0.04
4	B	0.2	6	0.06
5	B	0.4	10	0.10
6	C	2.0	29	0.29
7	C	2.0	48	0.48

The timer on the ion analyser is set to 5 minutes and is started immediately after the addition of each volume of standard solution. The mV value displaced on the meter is noted down immediately the timer sounds and the next addition is made. The timer is reset and the process repeated as necessary.

APPENDIX VIII

Composition of TISAB solution

The TISAB solution was prepared by mixing:

57ml glacial acetic acid
58 gm NaCl
4 gm CDTA (Cyclohexylene dinitrilo tetra acetic acid), with
500 ml of distilled water.

5M Sodium hydroxide was then added until the pH value of the solution was between 5.0 - 5.5. The final solution was made up to 1 litre by adding distilled water.

APPENDIX IX

Calculation of fluoride ion concentration in plaque

$$\text{ppm F/mg wet weight plaque} = \frac{\frac{M_{DIW}}{M_{PI}} \times \text{meter reading (ppm F)}}{M_{PI}}$$

This calculation assumes that the density of water is $1 g/cm^3$

APPENDIX X

Derivation of the equation by Angmar et al.(1963)

The grey level for any point in the lesion, which has resulted from x-ray absorption by both organic and inorganic components (thickness t_m and t_o respectively), can be equated against an equivalent aluminium (Al) grey level. Hence, for a particular level of absorption of x-ray (i.e. the grey value of a part of the lesion), the absorption can be equated against the absorption in an aluminium stepwedge.

Thus

$$u_a \; t_a = u_m \; t_m + u_o \; t_o$$

Where

u_a = linear absorption coefficient of the aluminium.

u_m = linear absorption coefficient of the mineral component.

u_o = linear absorption coefficient of the organic component.

t_m = thickness of the mineral element.

t_o = thickness of the organic element.

t_a = equivalent thickness of aluminium to give that grey value.

but $t_s = t_m + t_o$

where t_s = section thickness.

and

where V_m = volume of mineral component.

$$\frac{V_m}{V_s} = \frac{t_m}{t_s}$$

V_s = section volume.

thus

$$\frac{V_m}{V_s} \times 100 = 100 \frac{(u_a t_a - u_o t_o)}{(u_m - u_o) t_s}$$

The absorption coefficients depend on the radiation source (e.g. kV, target, filter, etc.) Angmar et al.(1963) employed CuK α radiation; u_a, u_m and u_a were found from known data and thus the equation reduces to :

$$\% \text{ Vol . min} = \frac{52.77}{t_s} t_a - 4.54$$

Thus the only unknown is t_a, since t_s can be measured. Therefore, for every point in the enamel, the equivalent aluminium thickness (t_a) is derived and percent volume mineral calculated.

APPENDIX XI

CONCENTRATION OF FLUORIDE IN PLAQUE (PPM/mg wet weight)
C1 = SUBJECT
C2 = TEST MATERIAL 1=VITREMER 2=DYRACT
C3 = PATTERN 1= TEST MATERIAL ON UR, LL CONTROL MATERIAL ON UL,LR
2 = TEST MATERIAL ON UL, LR CONTROL MATERIAL ON UR,LL
C4 = [F-] IN PLAQUE, UPPER TRIAL BEFORE BONDING
C5 = [F-] IN PLAQUE, UPPER TRIAL AFTER BONDING
C6 = [F-] IN PLAQUE, UPPER CONTROL BEFORE BONDING
C7 = [F-] IN PLAQUE, UPPER CONTROL AFTER BONDING
C8 = [F-] IN PLAQUE, LOWER TRIAL BEFORE BONDING
C9 = [F-] IN PLAQUE, LOWER TRIAL AFTER BONDING
C10 = [F-] IN PLAQUE, LOWER CONTROL BEFORE BONDING
C11 = [F-] IN PLAQUE, LOWER CONTROL AFTER BONDING

C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11
1	1	1	0.416	0.650	0.246	0.180				
3	1	1	0.846	0.796	0.698	0.247	1.262	5.619	5.888	11.983
4	1	1	6.822	5.224	0.225	5.207	0.353	15.196	1.616	1.449
5	1	1	0.079	0.066	0.139	0.346	0.059	6.980	0.052	1.050
8	1	1	2.410	10.987	1.628	0.416	0.756	1.100	0.483	1.184
22	1	1	0.810	5.190	2.340	0.950	0.200	1.600	0.280	0.120
24	1	1	5.780	2.940	0.240	0.004	0.390	0.130	4.950	0.140
2	1	2	4.923	2.070	1.353	1.860	1.387	3.924	4.294	0.214
6	1	2	0.520	1.399	0.697	3.878	2.261	11.183	0.230	2.986
7	1	2	0.234	4.280	4.259	1.805	1.415	3.365	1.100	8.019
11	1	2	1.220	11.050	0.810	3.830	0.640	0.760	3.010	2.274
16	1	2	0.210	0.430	0.390	0.640	0.190	4.800	0.160	0.460
21	1	2	1.580	10.590	0.450	0.550	1.000	0.730	12.950	5.210
10	2	1	1.960	2.240	2.770	0.210	4.640	9.720	2.980	0.780
15	2	1	1.240	3.540	4.880	0.920	0.760	5.260	0.920	1.350
19	2	1	0.030	28.700	0.720	11.600				
25	2	1	14.960	2.490	128.4	42.78	7.960	28.730	1.310	3.670
9	2	2	0.710	5.740	2.030	0.360	4.600	1.150	0.730	0.412
12	2	2	2.660	3.310	3.220	0.156	0.390	0.270	1.070	0.160
13	2	2	0.080	0.060	0.590	0.960	0.080	2.930	0.090	0.280
14	2	2	2.420	2.590	4.310	5.770	0.280	2.620	1.120	6.990
17	2	2	2.550	0.320	0.590	0.890				
18	2	2					0.570	0.220	0.670	0.090
20	2	2	1.190	1.110	0.250	0.550	0.220	6.540	0.870	4.920
23	2	2	61.600	0.230	22.900	0.960	5.580	0.470	29.200	0.370
26	2	2	0.860	0.089	0.349	0.340	0.141	0.305	0.038	0.694

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