AN IN VITRO COMPARISON OF TWO ORTHODONTIC BAND CEMENTS

by

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ABSTRACT

AIMS:

The aims of this in vitro study were to compare the fluoride release and antimicrobial properties of a light-cured hybrid glass-ionomer cement (Ultra Band-Lok, Reliance Orthodontics, Itasca, Ill., USA) with a conventional glass-ionomer cement (Ketac-Cem, Espe Oberbay, Gmbh). The pattern of microleakage when the two cements were used to cement orthodontic bands was assessed also.

MATERIALS AND METHODS:

Five discs (3.0mm diameter and 1.5mm thick) were prepared of each material. The discs were immersed in 2ml of deionised water which was changed daily. Measurements of fluoride concentration from the water were taken at regular intervals over a 40 day period using an Orion fluoride ion-selective electrode.

Antimicrobial properties were determined using an agar diffusion test. Unset cement was placed into 5mm wells in freshly prepared tryptic soy agar plates, two wells for each material per plate. The plates were inoculated with one of four different strains of Streptococcus mutans (NCTC 10499; Glasgow Dental Hospital strains (GDH) 96/1743; 96/1821; 96/1143). Six wells of each cement were tested for each bacterium. The plates were then incubated for 24 hours at 37 ^{0}C in a microaerophilic atmosphere. The zone of inhibition associated with each test cement well was taken as a measure of its antimicrobial activity against each Streptococcus mutans.

To assess microleakage, 15 bands were cemented with each material to intact caries-free third molar teeth. After storage in deionised water at 37°C for 1 month, specimens underwent thermocycling (850 cycles at 5, 37 and 50°C with a dwell time of 10 seconds), followed by immersion for 12 hours in 2% methylene blue dye buffered to pH 7. Microleakage was assessed by two examiners independently using a photographic record of an occluso-cervical section through the mesiobuccal groove of each tooth and a microleakage index. A random sample of 12 photographs were re-examined two weeks after the initial assessment to give an indication of inter- and intra-examiner reliability.

RESULTS:

The cumulative and daily fluoride release for days 5, 15 and 40 was significantly greater for Ketac-Cem than Ultra Band-Lok (Mann Whitney U test, $p < 0.05$).

The antimicrobial activity of Ketac-Cem was significantly greater for Ketac-Cem than Ultra Band-Lok over a 24 hour period for all four strains of Streptococcus mutans (Mann Whitney U test $p < 0.05$).

Intra-examiner (Kappa = 0.84 and 0.92) and inter-examiner (Kappa = 0.71) reliability were substantial when assessing microleakage. There was no significant difference (Mann-Whitney U test, $p > 0.05$) between the two cement groups for the enamel/cement interface ($p=0.657$) but a borderline significance was detected for the band/cement interface ($p=0.051$).

CONCLUSIONS:

The cumulative and daily fluoride release from Ketac-Cem was greater than for Ultra Band-Lok at days 5, 15 and 40.

The antimicrobial activity, over the first 24 hours, for Ketac-Cem was greater than for Ultra Band-Lok against the four types of Streptococcus mutans tested.

There was no significant difference in microleakage between Ketac-Cem and Ultra Band-Lok at the cement/enamel interface but there was a suggestion that there may be more microleakage with Ketac-Cem at the cement/band interface than with Ultra Band-Lok.

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DECLARATION

This is the original work of the author

CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

INTRODUCTION

Bands have been used in orthodontics for over 100 years. Since the introduction of the acid-etch technique (Buonocore, 1955) and the use of resin to bond orthodontic attachments (Newman, 1967), there has been a steady decline in the use of bands on anterior teeth. Bands, however, remain the attachment of choice for molar teeth where the occlusal forces are greater than anteriorly in the mouth and conditions are less favourable for bonding.

Glass-ionomer cements have been shown to be superior to non-adhesive cements for retaining bands (Maijer and Smith, 1988; Stirrups, 1991). They also have a number of beneficial characteristics including fluoride release (Forsten, 1977) and antimicrobial activity (DeSchepper, 1989a) which reduce enamel demineralisation under and around the band (Maijer and Smith, 1988; Rezk-Lega, 1991a). Conventional glass-ionomer cements are, however, prone to early moisture contamination and take 24 hours to reach their maximum strength (Wilson et al., 1979). In the past decade hybrid glass-ionomer cements have been introduced (Antonucci, 1988) in an attempt to address these problems.

This study is designed to compare two cements used for cementing orthodontic bands, Ketac-Cem, a conventional glass-ionomer cement in comparison to a new light-cured

hybrid glass-ionomer cement, Ultra Band-Lok. Previous studies comparing Ketac-Cem with Band-Lok a dual-cured hybrid glass ionomer of very similar composition to Ultra Band-Lok, have shown that both materials are comparable in terms of band retention and failure rate both in vitro (Millett et al., 1996) and in vivo (Fricker, 1997). This study aims to assess their fluoride releasing ability, their antimicrobial activity as well as to compare the position and extent of microleakage when bands are cemented with one of the two cements.

The current literature has been reviewed in the following sections. Section 1.1. discusses the development of orthodontic bands and band cements, including conventional glass-ionomer and hybrid glass-ionomer cements.

Section 1.2 discusses the role of fluoride in demineralisation, remineralisation and its effects on oral bacteria. Numerous methods have been used to assess fluoride release from dental cements and section 1.2 lists methods of detection and describes in detail the fluoride ion-specific electrode and the disc model used in the study to be reported here.

Antimicrobial activity has been shown to exist with conventional and hybrid glass-ionomer cements (DeSchepper, 1989; Souto, 1994). Only one previous orthodontic study has assessed the antimicrobial properties of orthodontic banding materials (Pavic et al., 1990) but a comparison between conventional and hybrid glass-ionomer cements for orthodontic banding has never been undertaken. Section 1.3 reviews the antimicrobial properties of glass-ionomer cements and discusses in vitro and in vivo methods of their evaluation. The agar diffusion test used in this study to assess the antimicrobial properties is described in detail.

The position of failure with orthodontic bands cemented with Ketac-Cem and Band-Lok has been shown to be different (Millett et al., 1996; Fricker, 1997), although no previous work has assessed this with regard to their microleakage pattern in relation to orthodontic bands. Section 1.4 reviews microleakage with conventional and hybrid glass-ionomer restorative cements, microleakage with crowns cemented with glass-ionomer cements and describes methods of assessing microleakage with special reference to the dye penetration method used in this study.

LITERATURE REVIEW

1.1 DEVELOPMENT OF THE ORTHODONTIC BAND AND ORTHODONTIC BAND CEMENTS

This section will discuss the development of the orthodontic band and review the cements used to retain them.

1.1.1 DEVELOPMENT OF THE ORTHODONTIC BAND

For hundreds of years, orthodontic tooth movement has been carried out with the first descriptions dating back to a few years B.C.. P. Fauchard, the founder of modern fixed appliance orthodontic treatment first described his 'bandalette' appliance in 1728, which enabled alignment of teeth to be achieved crudely by means of dental arch expansion. The use of a 'metallic' box or crown, carrying a tube attachment, moulded to the form of a tooth was described by Delabarre in 1819. Subsequent modification by Bell (1829) involved gold capping, swaged over the molar teeth, followed by adjustment at the gingival contour to allow engagement of the cervical crown undercut. In 1841, Schange invented a band that was fitted to the teeth by means of a clamp which was adjustable to accommodate different tooth size. The bands were constructed of gold, silver, copper and brass. Allen (1850) then described the plain band for use on incisor teeth to which Evans (1853) advocated the use of occlusal extensions preventing subgingival band migration.

Magill in 1871 first used dental cement to attach a band to a tooth. The clamp band was then modified by Angle (1913) and these bands were held in place by the clamp mechanism and dental cement. Plain bands made from silver or non-corrosive metal by either an indirect method or directly on a tooth in the mouth continued to be used, the overlapping metal joints and attachment being soldered. In the recent past seamless bands have been produced commercially and attachments are welded at the time of band fabrication.

1.1.2 CEMENTS USED TO RETAIN ORTHODONTIC BANDS

Although this study is primarily concerned with a conventional glass-ionomer cement and a light-cured hybrid glass-ionomer cement, other cements that have been used to cement orthodontic bands and brackets will be considered first. Twenty years ago it was common place for all teeth to be banded as part of fixed appliance therapy but nowadays only first molar teeth are banded routinely.

Cements, including gutta-percha (Bennett and Chapman, 1914), zinc oxide (Schroeder et al, 1974,) and zinc oxide eugenol (Williams et al., 1965) have been used to cement orthodontic bands but have not been widely accepted due to their poor compressive strength and lack of retentive properties. A consistency of cement required for orthodontic band cementation has been described by Cameron et al. (1963) and Craig (1985) as lying between that required for luting and cavity base purposes. Cameron et al. (1963) identified clinically used powder liquid ratios for orthodontic band cementation and using a slump test (B.S. : 3364 1961, A.D.A. Specification No.8)

identified a cement consistency producing a disc diameter for zinc phosphate of 26.5 ± 1mm to be optimal; similar work on glass ionomer/glass polyalkenoate cement has found disc diameters of 38mm (Ketac-Cem) to be ideal (Prosser et al., 1984).

The properties of a luting cement obviously have a bearing on the retention of an orthodontic attachment. The properties of three categories of cement (phosphate-based, polyacrylate-based and resin-based) will be reviewed.

1.1.2.1 Phosphate-based Cements

Zinc Phosphate Cement

Since its development, this cement has been used widely for cementation of orthodontic bands. Pattenbarger et al. (1933) carried out the first significant investigation into the composition of zinc phosphate cement. More recently in an attempt to protect the underlying enamel surface these cements have had high percentages of fluoride added e.g. 10 per cent stannous fluoride.

Zinc Hydrophosphate Cement

The mixing of dehydrated dihydrogen phosphate of zinc, calcium and other materials with water was aimed at improving the properties of zinc phosphate cements, but this proved to be of little benefit (Smith, 1983). Partial hydration of the powder resulted in very variable physical properties of the powder (Simons et al., 1968) and their

compressive strength, film thickness, solubility and disintegration may be inferior to other cements in this group (Craig, 1985).

Zinc Silicophosphate Cement

These cements have been available for a number of years and have been used extensively in laboratory and clinical studies of orthodontic bands. They were developed in the hope of combining the properties of zinc phosphate with silicate based cements. Zinc and magnesium oxide and silicate glass comprise the powder; the silicate glass contains 13.25 per cent fluoride.

Copper Cements

Copper cements have been used for cementing orthodontic bands for a long time, their main advantage being their germicidal effect. The powder is a zinc oxide with cuprous oxide or cupric oxide added. The liquid is similar to that used with zinc phosphate cement. Studies by Houston and Miller (1968) and Seeholzer and Dasch (1988) found this cement to have inferior properties to zinc phosphate cement and a glass-ionomer cement respectively.

1.1.2.2 Polyacrylate-Based Cements

Zinc Polycarboxylate Cement

In the 1960's, Smith developed the zinc polyacrylate/zinc polycarboxylate cements to produce a cement with other adhesive and biologically compatible properties while maintaining the physical properties of zinc phosphate cement. Zinc oxide is the main constituent of the powder with up to 10 per cent magnesium oxide or tin oxide added (Bertenshaw and Combe, 1972). In an attempt to improve strength, silica, alumina or stainless steel fibres have been added. In addition, stannous fluoride (4-5 per cent) is included in some brands. The liquid is composed of polyacrylic (32-42 per cent), itaconic and tartaric acids. However, some brands exist where the liquid is chiefly water with the polyacrylic acid coated onto the oxide particles in the powder. Heagney (1974) found this cement to be better than black copper cement for retaining orthodontic bands.

Glass Polyalkenoates (Glass-Ionomer Cements)

Wilson and Kent in 1972 were the first to describe glass polyalkenoate cements. Their physical properties were an amalgamation of those of silicate and polycarboxylate cements, but handling characteristics were not ideal. Lengthy setting times resulted from the reactivity of the aluminosilicate glasses in the early cements leading to poor wear resistance and high water absorption and solubility (McLean et al., 1984; Atkinson

and Pearson, 1985). The current glass-ionomer cements were introduced following the development of ion-leachable glasses (Wilson and Kent, 1972) and have better physical and clinical handling properties (Knibbs et al., 1986a, 1986b).

These cements are presented as a powder-liquid combination. Two types of conventional glass-ionomer cement exist - the conventional glass-ionomer consisting of an ion-leachable glass and a polyacid copolymer, or the water-hardening glass-ionomer consisting of a powder containing both the glass and a freeze-dried polymer. Ketac-Cem used in the present study belongs to this latter group. Mixing the two components of the conventional type or the addition of water in the water-hardening type activates the setting reaction.

Aluminosilicate glass with a high fluoride content (Prosser et al., 1982) forms the basis for the powder in glass-ionomer cements. It is formed by the fusion of quartz, alumina cryolite, fluoride, aluminium trifluoride and aluminium phosphate (Crisp and Wilson, 1976; Walls, 1986). Barry et al. (1979) have shown that two phases exist in the glass, a continuous calcium aluminosilicate matrix and partly crystalline particles rich in calcium and fluoride. The glass fusion temperature influences both the nature of the droplets and the final chemical composition. Non-matrix forming inclusions, (e.g. small metallic particles) have been incorporated in the powder to produce current cements with improved physical properties suitable for restorations (McLean and Gasser, 1985). The liquid in glass-ionomer cements is usually a 50 per cent aqueous solution of polyacrylic acid. Itaconic acid or maleic acid co-polymers were added later in an attempt to improve the shelf life by preventing gelation which occurred with the

early glass-ionomer cements. More recently tartaric acid has been added to the cement liquid to increase the rate of the setting reaction whilst maintaining an adequate working time. The polyacid is essential for chemical adhesion between dental tissue and cement, through the development of an ion enriched layer between cement and tooth tissue (Mount, 1994).

The alumino-silicate glasses used in glass-ionomer cement formulations contain droplets rich in calcium fluoride, the quantity being dependent on the fusion temperature of the glass (Barry et al., 1979). Analysis of the eluates of glass-ionomer cement have shown fluoride ions to be present (Crisp and Wilson, 1977; Crisp et al., 1974a; Matsuya et al, 1984). Their release continues after other ions have ceased to be leached (Crisp et al., 1974; Forsten, 1977). Fluoride uptake by hydroxy apatite has been found to be greater from glass-ionomer cement than from silicate cements over a seven week period in vitro (Forsten, 1977). In addition fluoride uptake by enamel adjacent to Class V glass-ionomer restorations has been shown to be retained for three to six months (Retief et al., 1984).

Clinical trials have shown conventional glass-ionomer cement to be more effective in retention of molar bands than non-adhesive zinc phosphate cement (Fricker, 1987; Stirrups, 1991).

Hybrid Glass Polyalkenoates

In an attempt to improve the properties of conventional glass-ionomer cements, various inorganic and organic additions have been made to the acid or base components. One

of the more recent additions is the incorporation of small quantities of liquid resin as used in conventional resin composites (Antonucci, 1988). This allows a sharper set and therefore reduces moisture contamination. In their simplest form the setting reaction of these hybrid materials involves an acid-base curing reaction between glass, tartaric acid and (poly)acrylic acid which is supplemented by a second curing process of a methacrylate, initiated by light. In these cements, the water component is replaced by a water/HEMA (hydroxyethylmethacrylate) mixture. HEMA differs from the hydrophobic methacrylates by the addition of a hydroxyl group that makes it hydrophilic (Wilson, 1990).

Hammesfahr (1994) has classified these hybrid glass-ionomer cements into: (1) materials containing a monomer, polymer and reactive glass; (2) materials containing monomer, polymer, hydrophilic group, polyacid, reactive glass and water; (3) materials where a methacrylate group is added to the polyacid with reactive glass and water and (4) materials containing smaller acid molecules on which a polymerizable methacrylate based group is added, reactive glass and water. Ultra Band-Lok, like its predecessor Band-Lok, appears to belong predominantly to the first group which tend to behave more like composite resins than true glass-ionomer cements. Due to their lack of polyacid and therefore their lack of an acid/base reaction it is questionable as to whether this type of material should be classified as a hybrid glass-ionomers cement (McClean et al., 1994). Phijiasanit and Tyas (1997) have suggested that these materials should be called light-cured resin composites with glass filler. For the puposes of this study, in view of the controversy over terminology Band-Lok and Ultra Band-Lok will

be grouped under the broad title of hybrid glass-ionomer cements.

The acid-base reaction when present in these hybrid materials is much slower than in conventional glass-ionomer cements because the water has been replaced by a water/HEMA mixture. However, a sharp set can be initiated with intense light allowing for rapid strength development, which has been found to be greater than that of conventional glass-ionomers (Compton, 1992; Burgess et al., 1993).

Chemical adhesion of hybrid glass-ionomer cement containing resin and glass ionomer components to dentine and enamel appears to be similar to that of conventional glass-ionomers (Lin, 1992) and like conventional glass-ionomer cements is dependant upon the presence of some polyacid (Mount, 1994). Ultra Band-Lok, if similar in composition to Band-Lok does not contain polyacid and its adhesion to enamel is principally mechanical as with resin composites (Phijaisanit and Tyas, 1997).

1.1.2.3 Resin-Based Cements

These cements have been used for cementing orthodontic bands since the 1970's (Lee et al., 1974) and are now used routinely to bond orthodontic brackets. For banding, this cement consists of a liquid/powder combination with a citric acid conditioner, to be applied to enamel for 2 minutes prior to band cementation. The liquid consists of diacrylate resin and the powder is composed of a filler to buffer changes in pH and a catalyst. A dry field, surprisingly, was reported not to be critical to good adhesion with this material (Sadowsky and Retief, 1976).

Setting of this cement is produced by polymerisation of the diacrylate monomer and contact with the initiator, usually an organic peroxide, which in the presence of a catalyst, produces a polymeric matrix which binds the hard filler particles. However, the short working times and difficulty in removing the cement from both the tooth and the band, largely exclude the use of these materials for routine cementation of orthodontic bands.

1.2 FLUORIDE RELEASE FROM ORTHODONTIC BAND CEMENTS

This projects aims, in part, to evaluate fluoride release from two orthodontic band cements. The role and mechanism of fluoride in the prevention of demineralisation, in the process of remineralisation and its inhibitory effects on cariogenic bacteria, together with fluoride release studies from orthodontic band cements and their methodology are outlined here.

1.2.1 ROLE OF FLUORIDE IN DEMINERALISATION:

The beneficial effects of fluoride in inhibiting dental caries has been known for many years, the predominant view of the early workers in the field being that it reduces enamel solubility by its incorporation into the apatite lattice. The fluoroapatite lattice formed is more stable and less soluable than the hydroxyapatite lattice (Brown, 1977). More recent studies, however, have shown that the levels of fluoride in the enamel do not necessarily correlate with levels of demineralisation (Larson, 1977; Retief, 1987). This is clearly demonstrated by Ogaard et al. (1988d) who showed that carious lesions formed even in shark enamel despite being almost pure fluoroapatite. Wong (1987) assessed apatite-bound fluoride, adsorbed fluoride and fluoride in solution on demineralisation and found that trace amounts of fluoride in solution are equally effective in decreasing the rate of demineralisation as compared to fluoride incorporated into the apatite lattice. The levels of fluoride in solution needed for this

reduction in demineralisation appeared to be in the order of 0-250μmol/l (Ten Cate, 1990), confirming the suggestion by Groeneveld (1990) that it is low levels of fluoride in drinking water that exert the main effects on inhibiting demineralisation in fluoridated areas rather than its incorporation into developing enamel.

It is better, therefore, to aim for a low maintenance dose of fluoride in the tooth environment than to enhance the concentration of fluoride in the tooth mineral in an attempt to reduce demineralisation. Zachrisson (1975) advocated daily use of a low fluoride concentration mouthwash (0.05% fluoride mouthwash) for patients undergoing fixed appliance orthodontics and Geiger (1988) suggested that this concentration of mouthwash could significantly reduce the incidence of decalcification on the labial surface of teeth during fixed appliance treatment.

Other methods of releasing low levels of fluoride into the oral environment as well as regular use of a fluoride dentifrice have also been attempted in orthodontics. These include removable appliances where calcium fluoride is blended into the acrylic (Miethke, 1988), a fluoride-releasing insert placed in removable appliances (Cooley and McCourt, 1991), fluoride-releasing elastic chain (Joseph, 1993) and fluoride-releasing retainers (McCourt et al., 1992).

In the past decade much interest has focused on fluoride-releasing cement particularly glass-ionomer cements for attaching orthodontic brackets and bands. Studies have found that areas of enamel adjacent to these materials undergo less demineralisation than those next to non-fluoride releasing materials (Valk and Davidson, 1987; Ogaard et al., 1992; Dubroc, 1994; Kindelan, 1996). Glass-ionomer cements used to cement

orthodontic bands have been shown clinically to reduce demineralisation beneath and around the band margins (Maijer and Smith, 1988; Rezk-Lega, 1991a). Tanaka (1987), using restorative glass-ionomer cement, found that the effect was somewhat localised. A further benefit of glass-ionomer cements is the possibility of fluoride uptake and release with the cement acting as a fluoride reservoir (Walls, 1986). This suggestion has been confirmed experimentally for both conventional and hybrid glass-ionomer cements (Forsten, 1991; Takahashi, 1993; Ashcraft et al., 1997).

1.2.2 THE ROLE OF FLUORIDE IN REMINERALISATION

Remineralisation is the process whereby partly dissolved crystals are induced to grow by precipitation of mineral ions from solution so that the process of mineral loss is to some extent reversed (Shellis and Duckworth, 1994). At neutral pH saliva and plaque fluid are supersaturated with respect to hydroxyapatite and can support remineralisation. Caries forms when the rate of demineralisation exceeds the rate of remineralisation (Margolis and Moreno, 1992).

The remineralisation process by which hydroxyapatite crystals are reformed occurs via intermediates, particularly octocalcium phosphate. Octocalcium phosphate then hydrolyses slowly to hydroxyapatite (Nancollas, 1974). Fluoride has been found to accelerate this hydrolysis and is incorporated in the product of crystal growth which in turn is less susceptible to dissolution (Amjad, 1979). The fluoride released from glass-ionomer cements has been implicated in the remineralisation of demineralised zones adjacent to bands cemented with conventional glass-ionomer cements (Althaus and

Donly, 1995).

Although fluoride in low concentration may be beneficial for remineralisation of demineralised areas, high concentrations of fluoride in the environment may in fact inhibit remineralisation by rapid crystal growth. This rapid crystal growth on the surface of a demineralised lesion blocks the surface pores leading to the lesion becoming arrested, rather than allowing remineralisation of the deeper layers of enamel (Ten Cate, 1990). High concentration of fluoride do, however, lead to precipitation of CaF_2 which because of its relatively high solubility dissolves into the local environment elevating the levels of fluoride locally (Arends, 1990).

1.2.3 THE EFFECTS OF FLUORIDE ON ORAL BACTERIA

This topic will be discussed more fully under the antimicrobial effects of glass-ionomer cements in section 1.3. In summary, the most important effect of fluoride in relation to oral bacteria is its action in reducing acid production (Kashket, 1977) which occurs even in low concentrations (Ferretti, 1982). The mechanism for this may be related directly to its action on the glycolytic enzyme enolase (Brown, 1977) or more likely, indirectly via its effect on hydrogen ion exporter system (Kashket, 1985). In addition there may be an effect on the ecology of the dental plaque (Marsh, 1990). The plaque itself appears capable of acting as a storage medium for fluoride whether in a bound or unbound form. Birkeland (1976) found the concentration of free fluoride in plaque increases as the pH falls and thereby becomes available for reducing demineralisation.

Hallgren (1993) found elevated levels of fluoride in plaque adjacent to glass-ionomer retained orthodontic appliances.

1.2.4 FLUORIDE RELEASE FROM ORTHODONTIC BAND CEMENTS

Glass ionomer cements have been shown to release fluoride over an extended period (Swartz, 1984). The rate of fluoride release from glass-ionomers is dependent upon various factors including; the amount of fluoride available in the material (DeSchepper, 1991), powder to liquid ratio (Muzynski et al., 1988), structure of the matrix (Fukazawa, 1987) and the effect of mixing time (Swift, 1988). In general, there is maximum release in the first 24 hours, with a large variation between different products (Miller, 1995). Fluoride release from hybrid glass-ionomer cements is dependant upon the acid/base reaction of the glass-ionomer components (Gasser, 1994) and is largely product dependent (Chadwick and Gordon, 1995; Wyness and Sheriff, 1996; Ashcraft et al., 1997; Monteith, 1997). It has been suggested that the fluoride release from these materials may be inhibited by the resin component (Wilson, 1990) although this has been disputed by the results of others (Mitra, 1991; Momoi and McCabe 1993) and in general their pattern of fluoride release appears similar to that of conventional glass-ionomer cements (Monteith, 1997).

Table 1.1 and Table 1.2 present an outline of studies where fluoride release and its associated effects have been determined for Ketac-Cem and Band-Lok. These studies show that Ketac-Cem and Band-Lok are capable of releasing significant quantities of fluoride (Muzynzki et al., 1988; Ashcraft, 1997) which in the case of Ketac-Cem

Table 1.1 In Vitro Studies Using Ketac-Cem, Measuring Fluoride Release and Associated Effects:

Wyness and Sheriff (1996)	Ketac-Cem (Glass ionomer) Dyract (Resin Modified Glass ionomer cement) EX#169 (resin modified glass ionomer cement) Rely-a-Bond (fluoride exchanging resin) Retain (composite control)	16 (2 x8 materials)	Distilled water or distilled water with TISAB(total ionic strength adjustment buffer)	Not stated	85 days	not stated.	1.Ketac-Cem released the most fluoride followed by EXM~169, then Dyract. 2. The fluoride-exchanging resins exhibited poor fluoride release. 3. The addition of TISAB accelerated the initial rate of fluoride release from those materials releasing significant amounts of fluoride.
Kindelan (1996)	Ketac-Cem (glass ionomer cement) Concise. (restorative composite) Concise plus Duraphat. Bondfast (composite bonding resin). Rely-a-Bond (fluoride leaching composite resin) Pulpodent O.B.A. (fluoride leaching composite resin.)	60 teeth (10 teeth for each material)	5ml demin solution pH 4.8.	Premolar teeth with brackets bonded.	4 weeks in demineralising solution.	Not relevant	Phosphorus levels disolved in solution were used to evaluate the levels of demineralisation. Ketac-Cem, Concise with Duraphat and Pulpodent O.B.A. performed significantly better in resisting enamel demineralisation than Concise alone, Bondfast or Rely-a-Bond.

Table 1.1 In Vitro Studies Using Ketac-Cem, Measuring Fluoride Release and Associated Effects:

Fox (1990)	Ketac-Cem (Glass ionomer luting cement)) Right -On (no-mix orthodontic bonding composite control) Direct (Orthodontic bonding composite)	30 specimens (10 x 3)	10ml of deionised water	square disc dimensions of 10 x 10 x 1mm	20 weeks	- At 2 days: Ketac-Cem 180ug Right On 5.8ug Direct 20ug At 20 weeks: Ketac-Cem 360ug Right On 12ug Direct 68ug	1. Ketac-Cem released more fluoride than Direct which in turn releases more than Right On 2. Direct has a different pattern of fluoride release to Ketac-Cem
Rezk-Lega et al. (1991a)	Ketac-Cem (glass ionomer cement) Aqua Cem (Glass Ionomer Cement)	6 specimens	Compared: a) 10ml human saliva b) 10ml distilled water c) 10ml water + HCL d) 10ml albumen e) 10ml phosphate buffer	Discs 8.5mm diameter and 3mm depth	1hour	Ketac-Cem a) 1.55ppm b) 7.2ppm c) 7.07ppm d) 6.5ppm e) 5.3ppm. Aqua Cem: a) 1.4ppm b) 3.75ppm c) 2.92ppm d) 2.5ppm e) 2.34ppm	For both cements the fluoride release was greatest for the distilled water less into the phosphate buffer and albumen and least into saliva.

Table 1.1 In Vitro Studies Using Ketac-Cem, Measuring Fluoride Release and Associated Effects:

Muzynski et al. (1988)	Ketac-Cem, Biocem, Everbond, Fuji type 1 (glass ionomer cements)	3 test specimens for each group	Placed in artificial saliva	Standard MOD castings were cemented on standard dies	170 hours.	(In ppm) Ketac Cem 7.3 Biocem 6.8 Everbond 17.67. Fuji 1 18.97.	1. Glass ionomer cements as luting cements release significant quantities of fluoride. 2. Commercially available cements vary in their fluoride released. 3. Cements with lower powder to liquid ratios release more fluoride. 4. Fluoride released suggests that they may be effective in reducing caries.
Valk and Davidson (1987)	Ketac-Cem (glass ionomer cement) Concise (Bis-GMA adhesive	35 teeth, (7 groups of 5 teeth)	Artificial caries inducing solution (van Dijk et al, 1979)	Bovine central incisors with brackets bonded	3 weeks	Not relevant	1. Fluoride releasing glass ionomer cement protected a substantial area of uncovered enamel adjacent to the brackets. The non-fluoridated cement did not even protect the area beneath the bracket. 2. Topical (APF) following bonding did not contribute to protection against in vitro demineralisation. 3. Limiting the area of acid etching reduces the area of enamel damage when composite resins are used.

Table 1.1 In Vitro Studies Using Ketac-Cem, Measuring Fluoride Release and Associated Effects:

AUTHOR(S)	MATERIALS	SAMPLE SIZE	MEDIUM	SPECIMEN TYPE AND SIZE	OBSERVATION TIME	FLUORIDE RELEASE	CONCLUSIONS
Swartz et al. (1984)	Ketac-Cem, Fuji, Chembond, (glass ionomer luting cements). Fluorothin (silico-phosphate cement) Durelon (polycarboxy-late cement) Aspa, Fuji II. Ketac (glass ionomer restorative cements M.Q.(Silicate cement)	Minimum of six specimens for each cement.	3ml of distilled water	Discs 10mm diameter by 2.25mm	12 months	not stated.	1. The patterns of release for all the luting cements are essentially the same 2. The polycarboxylate luting cement, Durelon, at the end of 30 days was only about one tenth that of the other luting cements 3. Fluoride is available from glass ionomer cements for a prolonged period of time and in the same amounts as silicate cements. 4. The glass ionomer luting cements appear to leach fluoride in approximately the same amounts as did the restorative cements.

Table 1.2 In Vitro Studies Using Band-Lok, Measuring Fluoride Release and Associated Effects

STUDY	MATERIAL	SAMPLE SIZE	MEDIUM	SPECIMEN TYPE AND SIZE	OBSERVATION TIME	FLUORIDE RELEASE	CONCLUSIONS
Ashcraft et al. (1997)	Band-Lok (dual-cured glass ionomer cement) Zionomer (light cured glass ionomer cement) Geristore (light-cured glass ionomer cement) Concise (composite resin bonding cement)	40 extracted premolars and canines. (10 per group)	3ml of deionised water. On day 48 the teeth were exposed to Gel Cam (0.4% SnF_2) for 30 seconds	Brackets bonded with each test cement to individual teeth	55 days	Day 1: Band Lok 1.49ppm Zionomer 0.36ppm Geristore 0.49ppm Concise 0.11ppm Day 7: Band Lok 0.50ppm Zionomer 0.05ppm Geristore 0.04ppm Concise 0.02ppm Day 49: Band Lok 1.17ppm Zionomer 0.25ppm Geristore 0.32ppm Concise. 0.04ppm	1. Band Lok released significantly more fluoride than Zionomer, Geristore and Concise. 2. The Hybrid glass ionomers were essentially releasing no fluoride after 6 weeks. 3) The light cured glass ionomer cements showed the ability to act as a reservoir for fluoride ions.

appears capable of protecting a substantial area of enamel adjacent to the cement (Valk and Davidson, 1987; Kindelan, 1996).

1.2.5 DIRECT AND INDIRECT METHODS FOR DETECTION OF FLUORIDE

In vitro and in vivo models have been developed for detection of fluoride from restorative/orthodontic materials. These are listed and described briefly in Table 1.3 and in Table 1.4. Orthodontic studies where each has been used are identified. As the study to be undertaken as part of the project reported here, uses the disc model with a fluoride ion electrode for fluoride release assessment, this will now be described in detail.

1.2.5.1 Fluoride Ion Specific Electrode

The commonest method of fluoride release detection is direct measurement of fluoride released into solution. This can be made via a fluoride ion-specific electrode connected to a specific ion meter, as described by Cranfield et al. (1982). The method often involves the manufacture of pellets or discs of the test material, which are then placed in solution and measurement of the fluoride concentration of the solution is taken periodically.

Workers have attempted to make the method more clinically relevant by placing the materials to be tested in artificially restored extracted teeth (Olsen et al., 1989) or placing the test material in saliva (Rezk-Lega et al., 1991b) rather than in deionised water. Glass-ionomer cements have been shown to release more fluoride into the

Table 1.3 Direct and Indirect In Vitro Models for Assessment of Fluoride Release

METHODS OF ASSESSMENT OF FLUORIDE RELEASE	DESCRIPTION OF METHOD	PREVIOUS ORTHODONTIC STUDIES
Disc Model	Discs/Pellets are constructed of the test material and are immersed in a solution, into which fluoride diffuses. Samples of the solution are taken at regular intervals and are analysed with a fluoride ion electrode attached to specific ion meter. The electrode is calibrated in solutions of known fluoride concentration .	Cooley et al. (1989) Fox (1990) Chan (1990) Rezk-Lega et al. (1991) Ogaard et al. (1992) Lynch and Tay (1989) Newman and Rudolf (1994) Wiltshire and Janse van Rensberg (1995) Chadwick and Gordon (1995) Wyness and Sheriff (1996) Monteith (1997)
Bracket Bonded Teeth Model	A brackets is bonded with a test cement to a tooth which is immersed in solution. The solution is sampled at regular intervals	Chan (1990) Bishara (1991) Ghani et al. (1994) Ogaard et al. (1992)
Acid Etch Biopsy	A layer of enamel taken from a defined area and of known depth is removed, The biopsy is dissolved in acid and the acidified solution is measured for fluoride with a fluoride ion probe.	Chadwick and Gordon (1995)
In Vitro Caries Model	This indirectly determines fluoride release via its beneficial effect on inhibiting demineralisation.Teeth are sectioned and viewed under polarised light microscopy or phosphorus levels determined from solution used to calculate mineral loss	Valk and Davidson (1987) Eliades et al. (1992) Althaus and Donly (1995) Kindelan (1996)
Modified Cavity Model	Determines fluoride release and antimicrobial effects of freshly mixed materials which come into contact with a culture medium via a semipermeable membrane	None
Fluoride Uptake Studies	Fluoride release monitored indirectly via its uptake into the hydroxy appatite lattice. This is dissolved in acid and the fluoride levels determined via a fluoride ion specific probe	Akkaya et al. (1996)
Spectrophotometry	This method relies on a change in colour of a metal dye complex as it is bleached when it comes into contact with fluoride during ion exchange.	None

Table 1.4 Direct and Indirect In Vivo Models for Assessment of Fluoride Release.

METHOD OF FLUORIDE ASSESSMENT.	DESCRIPTION OF METHOD	PREVIOUS ORTHODONTIC STUDIES
Salivary Fluoride Assessment	The concentration of fluoride in whole saliva during treatment of patients with fixed appliances, retained with a fluoride releasing cement, is measured.	Hallgren et al. (1990) Arends et al. (1990) Ogaard et al. (1997)
Plaque Fluoride Assessment	Plaque is sampled adjacent to bonded or banded orthodontic attachments and fluoride levels assessed by a fluoride ion specific electrode.	Hallgren et al. (1993)
Banding Model	Indirect assessment of fluoride release by assessing demineralisation. Specifically designed bands are attached to teeth scheduled for extraction. A spacer is placed between band and tooth to allow plaque accumulation. Alternatively white spot formation may be assessed visually adjacent to cemented bands. Alternatively the effects of the cement on demineralisation may be visualised, usually by photograph, post treatment .	Ogaard et al. (1986; 1990) Kvam et al. (1983) Rezk-Lega et al. (1991)
Bonding Model	A modification of the banding model, producing plaque tunnels under bonded brackets, is created. An assessment of white spot formation adjacent to bonded attachments, is made.	a) Forss et al. (1991) b) Sonis and Snell (1989) Underwood et al (1989) Bishara et al (1991) Mitchell (1992) Millett et al. (1992) Turner (1993) Millett et al. (1994) Trimpeneers and Dermaut (1996) Banks et al. (1997) Marcusson et al. (1997)
Removable Appliances	This model consists of a removable applience where the test materials cemented to enamel blocks are recessed into the appliance to allow plaque accumulation. The enamel is then tested to assess microhardness.	Ogaard et al. (1988c,d) Ogaard et al. (1990) Ogaard et al. (1992)
Animal Models	Animal models allow specific control of intra oral conditions	Dubroc (1994)

deionised water than into artificial saliva (Mallakh and Sarkar, 1990; Saito, 1978). It is important to note, however, that artificial saliva is not ideal either as it takes no account of pellicle or plaque which act as diffusion barriers.

Manufacturing discs, placing them in solution and taking sequential readings from them is a practical method of measuring fluoride release and one that has been used frequently to measure fluoride release from orthodontic bonding (Cooley et al, 1989; Fox, 1990; Ogaard et al., 1992; Newman and Randolph, 1994; Wiltshire and Janse van Rensberg, 1995; Chadwick and Gordon, 1995; Monteith, 1997; Ogaard et al., 1997) and banding materials (Rezk-Lega et al., 1991b). There appear to be large variations in the details of each study in terms of sample size, observation time, units of measurement of fluoride, medium type, medium pH and quantity/dimensions of the materials tested. Cranfield et al. (1992) looked at the factors which determine the rate and extent of fluoride release including sample geometry. They found that for discs with the same surface area exposed, the larger the volume, the greater the amount of fluoride they released.

1.3 ANTIMICROBIAL EFFECTS OF ORTHODONTIC BAND CEMENTS

Part of this study involves an assessment of the antimicrobial properties of two orthodontic band cements. The following section will review the literature with regard to antimicrobial propertes of glass-ionomer cement and give an outline of the methods used to detect antimicrobial properties with special reference to the agar diffusion test which is the method adopted in this study.

1.3.1 ANTIMICROBIAL PROPERTIES OF GLASS-IONOMER CEMENTS

The presence of fixed orthodontic appliances interferes with both active and passive oral hygiene measures leading to increased plaque accumulation with a resultant rise in demineralisation (Zachrisson, 1974; Gorelick et al., 1982; Ogaard et al, 1988a). To prevent demineralisation in patients undergoing fixed appliance therapy there have been a number of preventive regimes recommended based on fluoride and fluoride-releasing agents, including the use of glass-ionomer cements for bonding and banding (Zachrisson 1975; Ogaard et al., 1988a).

Glass-ionomer cements have been shown to have antibacterial properties in vitro (Tobias et al., 1985; De Schepper et al., 1989a; Meryon and Johnson, 1989; Einwag et al., 1990; Palenik, 1992; Fischman and Tinanoff, 1994; Palenik and Setcos, 1995) and in vivo (Svanberg et al., 1990; Forss et al., 1991; Hallgren et al., 1992; Benelli et al., 1993). These antimicrobial properties have been found, however, to diminish rapidly in

sucrose solution (Seppa, 1992; Berg, 1990) but may be prolonged in vitro by the application of 1.23 % Fluoride gel (Seppa et al., 1992, 1995). Antimicrobial properties have also been demonstrated for hybrid glass-ionomer cements in vitro (De Schepper et al., 1989b; Souto, 1994; Friedl et al., 1997) and in vivo (Wright, 1996).

The antimicrobial properties of glass-ionomer cement are thought to be due to their low pH during their setting reaction and their acid concentrations have been reported to be well below those reported to be bactericidal (DeSchepper et al., 1989a). Fluoride release from glass-ionomer cements is also thought to play a role in their antimicrobial properties. Though the effect of the fluoride is unknown, it has been found to have an effect at low concentrations (20-300ppm), the sensitivity of oral bacteria to fluoride being increased with lowering of pH (Hamilton and Bowden, 1988). It has been known for some time that fluoride inhibits the glycolytic enzyme enolase but this appears to require high concentrations of fluoride >0.5mg/l (Cimasoni, 1972) which would be unlikely with the fluoride release from glass-ionomer cement. Fluoride also appears to inhibit the ATPase responsible for pumping hydrogen ions out of the bacterial cell (Kashket, 1985). This is the mechanism which enables the cell to maintain a near normal cytoplasmic pH. As a consequence of cytoplasmic acidification, fluoride produces indirect inhibition of acid production. Finally, fluoride may alter the ecology of dental plaque by removing the competitive advantage possessed by acidogenic, acidouric organisms such as Streptococcus mutans (Marsh and Bradshaw, 1990).

1.3.2 METHODS OF EVALUATING ANTIMICROBIAL ACTIVITY

Table 1.5 and 1.6 give a brief outline of in vitro and in vivo methods of assessing antimicrobial properties of test cements and orthodontic studies where they have been used are identified. As the agar diffusion test was used in the study reported here it will now be discussed in more detail.

1.3.2.1 Agar Diffusion Tests

Many investigations have been carried out into the antimicrobial action of dental cements. In his review of the literature, Tobias (1988) stated that the agar diffusion inhibitory test (ADT) is the most common method for assessing the antibacterial properties of dental materials.

Agar diffusion inhibition tests involve the application of a cement or material to a previously inoculated agar plate. This is then incubated and the plate is assessed for a zone of inhibition, the suggestion being that the larger the zone of inhibition the greater is the amount or strength of the bactericidal agent present. This method, however, has several drawbacks. It does not differentiate between bactericidal and bacteristatic effects, nor does it provide any indication as to the viability of the test organisms at the end of the experiment.

The size of the zone of inhibition reflects a number of factors relating to the solubility of the agents in the agar and their speed of release within the first few hours of placement which can directly effect the zones of inhibition.

Table 1.5 In Vitro Methods of Assessment of Antimicrobial Properties of Cements

METHOD OF ASSESSMENT	SUMMARY OF METHOD	PREVIOUS ORTHODONTIC STUDIES
Agar Diffusion Tests(ADT)	ADT involves the application of a test cement to a previously inoculated agar plate. The plate is then incubated and the plates are then assessed for a zone of inhibition. The zone of inhibition reflects the strength of the antimicrobial agent present.	Pavic et al. (1990)
Bacterial Adherence Assays	The specimens are dry weighed and then immersed in a freshly inoculated culture broth for a set time period. They are then removed and dry weighed. The weight gain is representative of the of the quantity of bacterial growth on the cement.	Blunden et al. (1994)
Bacterial Coverage Scores	The material is incubated in a freshly inoculated culture media; it is then washed gently and the adherent material is disclosed with a 0.075% (w/v) basic fuchsin solution. The material is scored for percentage coverage of the material and material thickness measured by stain intensity.	NONE
Nephalometrics	Nephalometric assessment involves the use of a photometer to determine the transmission of electromagnetic waves through a culture medium. From the transmission of the waves a density of bacteria can be determined.	NONE

Table1.6 In Vivo Methods of Assessment of Antimicrobial Activity of Cements

METHODS OF ASSESSMENT	SUMMARY OF METHOD	PREVIOUS ORTHODONTIC STUDIES
Plaque Assessment	plaque samples from around fixed and removable orthodontic appliances may be used to assess: 1) bacterial numbers 2) metabolite production	Bacterial numbers Hallgren et al. (1992) Wright et al. (1996) Metabolite Production Hallgren et al. (1994)
Saliva Assessment	Levels of cariogenic bacteria are estimated prior to insertion of fixed appliance and then after insertion and cementation with test cement or control.	Georgoudi et al. (1991)

Barry (1976) discussed the dynamics and variables associated with ADT. He suggested that contact of the test material with the gel, the molecular weight of the antimicrobial agent, the diffusion through the gel of the antimicrobial agent, the inoculum density of the test organism and the time of incubation influence the zone of inhibition. In addition, the agar medium (nutritive capacity, depth of the agar), the temperature of incubation (ideally 37^0C), the measurement of the inhibition zones (diameter or area) and the test micro-organism all play a role in varying the diameter of the zones of inhibition obtained.

1.3.2.2 Orthodontic Studies Using (ADT)

Only one previous study has used the agar diffusion test to assess antimicrobial activity of orthodontic band cements (Pavic et al., 1990). They assessed the antimicrobial properties of zinc phosphate, conventional glass-ionomer and polycarboxylate cements against Streptococcus mutans and Lactobacillus acidophillus. Their findings demonstrated variations in the antimicrobial properties of the materials studied. DeSchepper et al. (1989a), used a modified ADT method to compare the antibacterial effects of conventional glass-ionomer cements, including Ketac-Cem. They modified the basic ADT method, by creating wells in the agar using sterile punches, into which were placed the materials to be tested. This replaced the previous technique of placing test materials on the surface of the agar. It was the purpose of their study to test the antibacterial properties of several commercially available glass-ionomer formulations against Streptococcus mutans #6715. In addition to studying the mixed cements, the

antimicrobial activity of the individual components and lastly the effect of the fluoride release and pH of the cements were tested. Their conclusions were that the glass-ionomer cements tested inhibited the micro-organisms; antimicrobial activity was found in many of the components; the antimicrobial activity included that derived from the polyalkenoic compounds of the cements and the fluoride released from the setting reaction. Fischmann and Tinanoff (1994) attempted to determine the relationship between fluoride release and the antibacterial effects of four restorative glass-ionomer cements using an ADT on inoculated blood agar plates. Palenik and Setcos (1996) used the ADT method to compare dentine bonding agents and restorative materials. They compared direct and indirect inhibition of their test materials. The indirect inhibition test involved removing the test specimens from the plates after 48 hours at 37^{0}C and then inoculating the plates with a test micro-organism. Their results suggested that although some inhibition was detected via the direct inhibition test, none was detected for the indirect inhibition tests. Thus, the antimicrobial agents were not released in sufficient amounts in the absence of the material to inhibit bacterial growth.

1.4 MICROLEAKAGE WITH ORTHODONTIC BAND CEMENTS

In part, this project aims to evaluate microleakage associated with bands cemented with two orthodontic band cements. A review of the literature indicates that no previous study has examined microleakage between banded teeth and orthodontic banding cements. This section will, therefore, outline previous microleakage studies using conventional and hybrid glass-ionomer restorative cements; microleakage associated with crowns cemented with conventional and hybrid glass-ionomer cements and will outline methods of assessing microleakage with particular reference to the dye penetration method used in this study.

Microleakage is defined as "the clinically undetectable passage of bacteria, fluids, molecules or ions between a cavity wall and a material applied to it" (Kidd, 1976). Interest in microleakage with dental materials was initiated by a classic experiment performed in 1952 when Nelson et al., using materials available at that time, were able to demonstrate that temperature changes induced at the margins of the dental restorations resulted in percolation at the tooth restoration interface.

1.4.1 MICROLEAKAGE WITH CONVENTIONAL AND HYBRID GLASS-IONOMER CEMENTS USED FOR ORTHODONTIC BANDING

Previous in vitro work by Millett et al. (1996) and in vivo by Fricker (1997) have suggested that the position of bond failure, when comparing bands cemented with Ketac-Cem or Band-Lok, is largely mixed in nature. There is a tendency, however, with

Ketac-Cem for failure to be between band and cement whereas with the hybrid glass-ionomer cement Band-Lok, band failure is more common between cement and tooth. It has been suggested that there may be a path of microleakage between tooth and cement with bands cemented with Band-Lok, and that possibly this may lead to decalcification under the band without necessarily the band loosening (Fricker, 1997).

1.4.2 MICROLEAKAGE WITH CONVENTIONAL OR HYBRID GLASS-IONOMER CEMENT USED FOR RESTORATIVE PURPOSES

Conventional glass-ionomer cements undergo little shrinkage during setting (Feizler et al., 1988) and their coefficient of thermal expansion is similar to that of tooth structure (Albers, 1985). They are capable of chemically adhering to tooth structure (Hotz, 1977) via calcium bridges, hydrogen bonds, Van der Waal forces and most importantly "phosphate substitution" (Wilson, 1983). The addition of a resin component to the glass-ionomer cement, producing a hybrid glass-ionomer cement results in increased polymerisation shrinkage (Hinoura et al., 1992) possibly leading to disruption of the adhesive bonds between tooth tissue and the cement. It has been suggested that the contraction in the hybrid glass-ionomers may be compensated for by the flow characteristics of the glass-ionomer cement hydrogel matrix during maturation (Tsuenekawa, 1992) or by hygroscopic expansion (Irie et al., 1992). Results of microleakage studies on differing types of hybrid and conventional glass-ionomer restorative cements appear variable when these materials are bonded to enamel (Lim, 1990; Hallet and Garcia-Goodoy, 1997). In general, however, it appears that no

conventional or hybrid glass-ionomer restorative cement will completely resist microleakage (Alperstein et al., 1983; Thornton et al., 1988).

1.4.3 MICROLEAKAGE WITH CONVENTIONAL AND HYBRID GLASS-IONOMER CEMENTS USED FOR RETAINING CROWNS

Although no previous studies have looked at microleakage of banded teeth, studies have, however, compared microleakage associated with crowns cemented with glass-ionomer cement to that obtained with other luting cements (Tjan et al., 1991; White et al., 1994). Results of these studies suggest that cast gold crowns cemented with glass-ionomer cement suffer from significantly less microleakage than those cemented with zinc phosphate cement.

The results with accurately fitting cast gold crowns are probably less relevant to the present study than those that assess microleakage around preformed crowns where the fit is less accurate. When comparing glass-ionomer and hybrid glass-ionomer for cementing stainless steel crowns, Shiflett et al. (1997) found that there was no significant difference between the two cement groups, but that adhesive cements were capable of significantly reducing microleakage when compared to non-adhesive cements such as zinc phosphate, polcarboxylate and zinc oxide eugenol.

1.4.4 ASSESSMENT OF MICROLEAKAGE

A review of restorative studies related to microleakage shows that many and sometimes ingenious techniques have been developed to study marginal permeability at the

interface between tooth and restoration. These studies emphasize that the margins are not fixed, inert and impermeable, but are rather "dynamic microcrevices which contain a busy traffic of ions and molecules" (Myers, 1996). All methods that have been used to assess microlaekage are summarised in Table 1.7 together with their advantages and disadvantages. As the present study uses the dye penetration method for assessment of microleakage, this method and the means of assessment of the depth of dye penetration will now be described in more detail.

1.4.4.1 Dye Penetration Method

Dyes are one of the oldest and most frequently used methods of studying microleakage and represent a simple and inexpensive technique (Delivanis, 1983). As early as 1895, Fletcher, as reported by Blackwell, used dyes to study the shrinkage of amalgam. Fluorescent dyes were found to be particularly useful as tracers because they are detectable in dilute concentration and are easily photographed under visible light (Going, 1972). Eosin, methylene blue, methyl violet, haematoxylin and mercuric chloride, prontosil soluble red, aniline dye, basic fuchin, chromotope 2R, crystal violet and fluorescein are a few of the many dyes that have been used. These are simple to use, inexpensive, non destructive and demonstrate leakage without a chemical reaction or exposure to hazardous materials. They do, however, have limitations in the area of microleakage evaluation as no quantitative measurements can be obtained (Going, 1985).

Table 1.7 Methods for Assessing Microleakage

METHOD OF MICROLEAKAGE ASSESSMENT	SUMMARY OF METHOD	ADVANTAGES	DISADVANTAGES
Dye Penetration Method	Specimens are immersed in organic dyes, which percolate along the path of microleakage by capillary action and may be visualised under the light microscope, following sectioning	Simple Inexpensive Non hazardous Physical effect does not rely on chemical reaction. May be performed in vivo but requires tooth extraction.	No quantitative measurements are possible.Different dyes vary in penetration.Recordings may be subjective and teeth have to be sacrificed.
Autoradiography	Specimens are immersed in a radioisotope, most commonly calcium chloride which percolates along the path of microleakage by capillary action. Following sectioning, the specimen is placed on photographic emulsion producing a latent image.	Relatively simple. May be performed in vivo but requires tooth extraction.	Hazardous materials, choice of isotope, distance between source and emulsion and length of exposure all have an effect. It has been suggested that the calcium ion may react with hydroxy appetite crystals and no quantitative measurements are possible. The isotopes are water soluable and therefore have the risk of spread over uncontminated areas when sectioned. Recordings may be subjective and the teeth have to be sacrificed.
Chemical Tracers	The specimen is immersed in two colourless compounds separately that react to produce an opaque precipitate that may be detected	Relatively simple. Nonhazardous.	No quantitative measurement possible. It relies on a chemical reaction, the teeth have to be sacrificed and holds little advantage over dye penetration method.

Table 1.7 Methods for Assessing Microleakage

Bacterial and Bacterial Metabolites	The specimens are inserted in a culture of micro-organisms or their end products	More clinically relevant.	Complicated and no quantitative assessment is possible. The leakage from the bacteria may be less than H+ ions. It may, therefore, under estimate the true microleakage possible.
Air Pressure Method	Air under pressure is delivered from the base of the restoration	Allows quantitative assessment. Allows longitudinal study.	Not relevant clinically. Leakage is from the base not the occlusal surface and the technique works on the basis that the specimen leaks in a uniform manner.
Marginal Percolation	This uses thermodynamics to indicate fluid movement	Tooth does not have to be destroyed	No quantification of measurement and only assesses leakage from the surface.
Scanning Electron Microscope	The specimens undergo sectioning and various preparation methods to allow visualisation under the scanning electron microscope.	In vivo assessment possible,but only assessing the surface but will allow longitudinal monitoring.	Expensive, with no direct correlation between adaptation of test material and microleakage. There is a tendency to produce artefacts.
Neutron Activation Analysis	Permeation of manganese is allowed around the specimen margin. The uptake is assessed by a neutron activator.	Allows quantification of microleakage.	Expensive and the path and depth of penetration is difficult to define. It is a dangerous procedure relying on nuclear physicist.
Electrochemical Assessment.	Relies on the principal that an electro-galvanic current will flow between an anode and cathode when an electrical pathway is established due to microleakage.	Allows quantification of microleakage. Easily compared and analysed. Longitudinal study possible in vitro. Reduces laboratory preparation and mistakes.	Relatively complicated and it is not possible to perform with metal restorations.

Table 1.7 Methods for Assessing Microleakage

Artificial Caries.	Specimins are placed in a demineralising solution, usually a mixture of saliva and demineralising solution	Relevant clinically. Quantifiable.	The tooth is sacrificed and is complicated by the assessment of demineralisation.
Calcium Hydroxide Technique	Calcium hydroxide is placed at the base of a restoration and lithmus paper used to assess alkalinity at the surface of the restoration	Allows longitudinal assessment. Reliable, simple and biocompatible.	Quantitative and measuring from the base to the surface only.

Different techniques may be employed based upon dye immersion:

Passive dye penetration:

This works via capillary action and depends upon the hydrophobicity of the material and the tooth surface.

Centrifugation:

This increases the hydrostatic pressure of the dye and by compressing the air in the voids allows the dye to penetrate further.

Reduced Pressure Technique:

Goldman et al. (1989) suggested that any quantitative evaluation was meaningless unless entrapped air is evacuated from the voids. Many researchers, however, have failed to find any difference between passive dye penetration and reduced pressure techniques, (Dalat and Spanberg, 1994). It must also be questioned as to whether reducing the air pressure or increasing the hydrostatic pressure is relevant clinically.

1.4.4.1.1 Assessment of the Depth of Dye Penetration

Semi Quantitative Data:

Linear measurement is the most popular method, based on the supposition that linear penetration of the tracer indicates the extent of the gap.

Longitudinal Sectioning:

This method will not allow a three dimensional assessment of the leakage to be made, making the results difficult to extrapolate clinically.

Clearing Technique:

This allows the whole area to be examined, but methods of preparation in the technique may result in interference with the detection of the dye.

Quantitative Data:

Douglas and Zakariasen (1981) introduced a volumetric analysis by which teeth were immersed in 2% methylene blue dye and the excess dye was removed. Each tooth was then dissolved in dilute nitric acid which returned the dye to solution. This could then be assessed spectrophotometrically.

1.5 AIMS OF THE STUDY

1. To compare the fluoride-releasing characteristics of two orthodontic band cements, a light-cured hybrid glass-ionomer cement (Ultra Band-Lok) (Fig.1.1) and a conventional glass-ionomer (Ketac-Cem) (Fig.1.2).

2. To compare the antimicrobial activity of the two cements against four strains of Streptococcus mutans using an agar diffusion test.

3. To compare the position and extent of microleakage when the two materials are used to cement orthodontic bands to third molar teeth.

The following **null hypotheses** will be tested.

1. There is no difference between Ultra Band-Lok and Ketac-Cem with regard to their daily and cumulative fluoride release.

2. There is no difference between Ultra Band-Lok and Ketac-Cem in relation to their antimicrobial activity against the four strains of Streptococcus mutans tested.

3. There is no difference in the extent and position of microleakage when Ultra Band-Lok and Ketac-Cem are used to retain orthodontic bands.

Figure 1.1: Ultra Band-Lok (Reliance Orthodontics, Itasca, Illinois, USA).

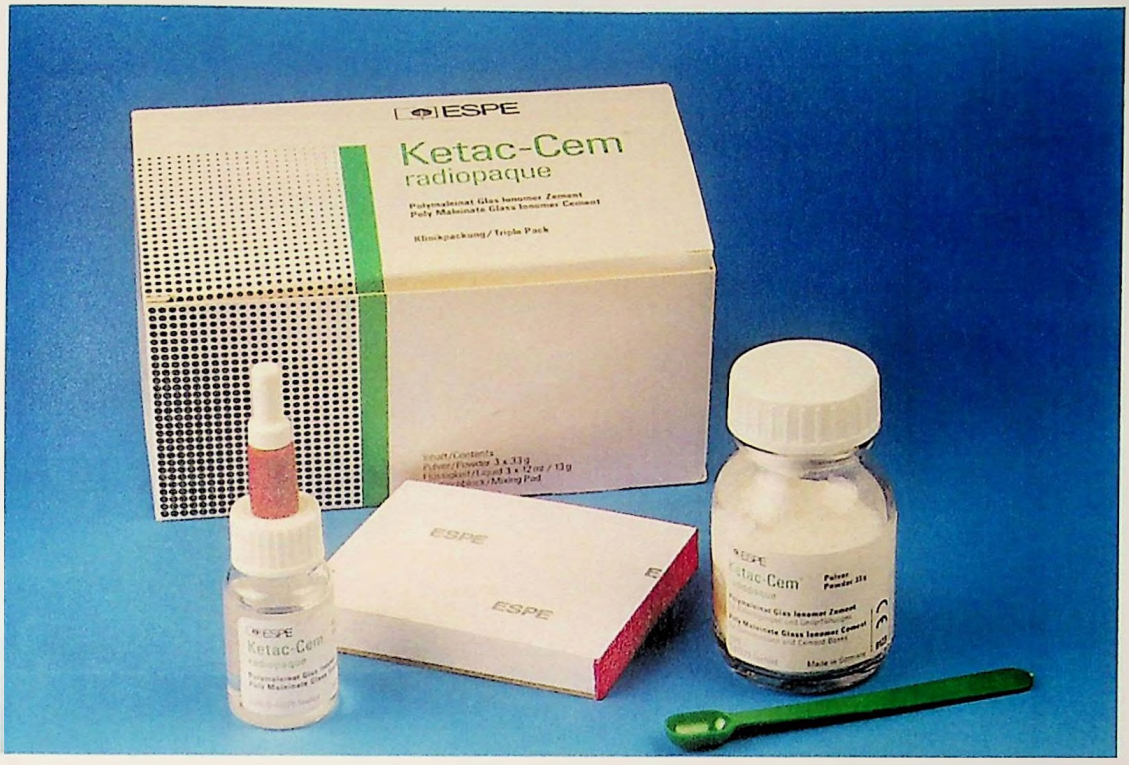

Figure 1.2 : Ketac-Cem (Espe, Gmbh Oberbay, Germany).

CHAPTER 2

MATERIALS AND METHODS

2.1 METHOD OF DISC SPECIMEN PREPARATION AND ASSESSMENT OF FLUORIDE RELEASE

2.1.1 DISC SPECIMEN PREPARATION

Each material was prepared according to the manufacturer's instructions with the operator wearing latex examination gloves (Microtouch, Johnson & Johnson Medical Inc. Arlington, Texas, USA) to avoid surface contamination of the test materials. Five discs of each type of cement were manufactured using a stainless steel mould 3mm diameter x 1.5mm depth (Fig.2.1). The cements were compressed into the stainless steel mould using two glass slides. The discs of Ketac-Cem (Espe, Gmbh Oberbay, Germany) were left to bench cure for 24 hours in a dry environment and the discs of Ultra Band-Lok (Reliance Orthodontics, Itasca, Illinois, USA) were light cured for 60 seconds using ESPE Elipar light (ESPE, Fabrik Pharmazeutischer Praparate, Seefeld, Germany).

The discs were then placed in plastic vials containing 2ml of deionised water. After 24 hours each disc was removed from the vial, blotted dry, using fresh filter paper, to prevent cross-over contamination and then immersed in 2ml of fresh deionised water.

Figure 2.1: 3mm by 1.5mm discs of Ketac-Cem

The deionised water solutions were changed on a daily basis for 15 days and then at 3 or 4 day intervals for a 45 day period. The solutions into which the fluoride had been released were then stored in a freezer at -20 degrees celsius until fluoride analysis could be carried out.

2.1.2 ASSESSMENT OF FLUORIDE RELEASE

The ionic fluoride concentration of each solution was measured using an Orion combination fluoride ion-selective electrode (Orion Research Electrode No 9609BN) attached to an analyser (Orion Research Ion Analyzer EA940, Boston Massachusetts, USA) (Fig. 2.2). This electrode has a lanthium trifluoride membrane which generates an electrical potential across the membrane when immersed in a solution containing fluoride ions. The difference in electrical potential across this membrane is dependant on the fluoride ion concentration of the test solution and is displayed as a digital reading (millivolts) on the ion analyser.

Fluoride estimations are pH dependent so it was necessary to buffer the solutions being analysed by mixing them with equal parts of total ionic strength adjustment buffer (TISAB) - see Appendix I. This standardises measurement conditions to between pH 5.0 and 5.5 and it also prevents drifting of potentiometric measurements by the electrode by increasing the ionic concentration of the test solution. As the solutions being measured were expected to contain less than 0.4ppm (parts per million) fluoride i.e. low fluoride concentrations, a low level TISAB solution was used. All solutions were allowed to reach room temperature before measurement and were stirred using a

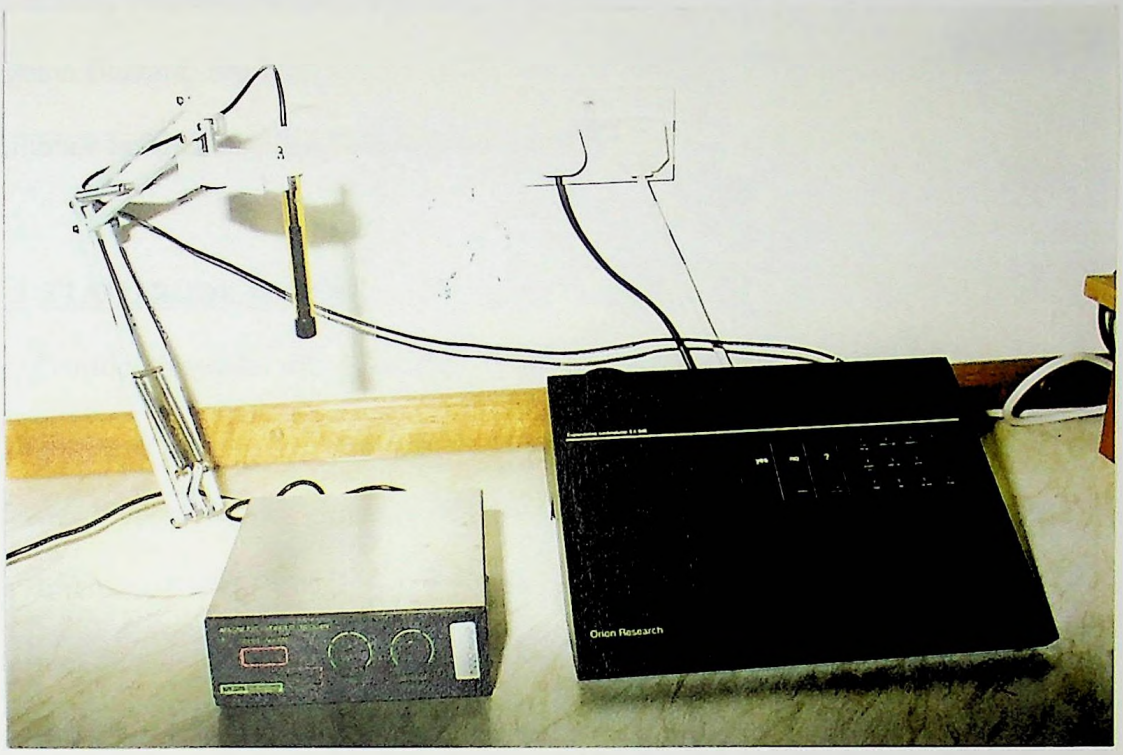

Figure 2.2: Orion Research Electrode (9609BN) positioned above a Magnetic Stirrer (H1304N) and connected to an ionanalyser (Orion Research Expandable Ion Analyzer).

non-heating magnetic stirrer (Magnetic Stirrer, H1304N, Jencons Scientific Limited, Leighton Buzzard, England) for a 5 minute period whilst the electrode was allowed to equilibrate before the millivolt reading was recorded.

2.1.3 ELECTRODE SLOPE:

The fluoride electrode was calibrated at the beginning of each measuring session (approximately 3 hours). The electrode was immersed in solutions of 10ppm fluoride and then 100ppm fluoride solutions, allowing 5 minutes for each reading to stabilise. The difference between these two readings was in the range of 55-60mV. This is known as the electrode slope and indicates that the electrode is in good condition and is responding properly.

2.1.4 LOW LEVEL CALIBRATION CURVE

To convert the millivolt readings to parts per million (ppm) of fluoride a calibration curve was produced prior to each measuring session. For fluoride concentrations of less than 0.4ppm the calibration curve becomes non-linear and this necessitates the preparation of a special low-level calibration curve.

A low-level TISAB solution was prepared by making up TISAB solution without adding CDTA (- see Appendix II). Fifty millilitres of low-level TISAB was then added to 50ml of 10ppm fluoride standard solution to produce a low-level standard solution. Increasing concentrations of the low-level standard solution were then added to a mixture of 50ml deionised water and 50ml low-level TISAB in incremental steps so that

a reading in millivolts could be obtained for known concentrations of the standard fluoride solution (- see Appendix III).

The readings for fluoride measurement in millivolts were then entered into a computer using a software programme which plots a calibration curve for fluoride measurement (mV) against fluoride concentration (ppm) for each sample (Fig.2.3). If the calibration curve was found to be acceptable (+/- 5% of the value for each point), the sample solutions were then measured and the readings in millivolts obtained could be processed by the computer to provide fluoride concentrations in ppm for each sample.

2.1.5 FLUORIDE MEASUREMENT OF INDIVIDUAL SAMPLES

One millilitre of the solution to be tested was added to 1ml of low-level TISAB in a small disposable plastic dish, stirring the solution continuously using a small magnetic stirring bar on a non-heating magnetic stirrer. Cling film was wrapped around the electrode and microsample dish to minimise evaporation during the measuring procedure and the electrode was allowed to stabilise for 5 minutes before recording the reading in millivolts.

Between measurements the electrode membrane was gently rinsed with deionised water, then dried to prevent cross-over contamination between samples. A sample of deionised water was also measured as a control for background fluoride.

The concentration of fluoride (ppm) in samples collected at the following time intervals-6 hours, 12 hours, 18 hours, 1-15, 20, 30, 40 days- was determined from the low-level calibration curve. For samples collected at 20, 30 and 40 days, the results

Figure 2.3: BBC Computer and Printer for plotting the fluoride callibration curve.

represented the cumulative fluoride release over a 3 or 4 day period and so these results were divided accordingly, to calculate the mean daily fluoride release.

2.1.6 STATISTICAL ANALYSIS

The daily and cumulative fluoride release totals for the two test cements were compared by means of a Mann Whitney U test.

2.2 METHOD OF TEST SPECIMEN AND AGAR PLATE PREPARATION AND ASSESSMENT OF ANTIMICROBIAL PROPERTIES OF TEST CEMENTS

2.2.1 TEST SPECIMEN AND AGAR PLATE PREPARATION

Twelve tryptic soy agar plates containing 15ml of agar were prepared in advance of each session, allowed to set and a sterile steel punch was used to create four holes of 5mm diameter in each plate to test the freshly mixed cements.

Streptococcus mutans: NCTC 10499; Glasgow Dental Hospital strains (GDH) 96/1743; 96/1821; 96/1143 were cultured on Columbia blood agar plates and a suspension of each Streptococcus mutans was made in sterile saline to give a density equivalent to Macfarland tube No.4.

The agar plates were inoculated by dipping a sterile swab in a freshly prepared culture of the test strain of Streptococcus mutans and then streaked over the plate surface in three directions. Each test strain of Streptococcus mutans was used to incubate 3 plates. Both the cements were prepared aseptically, the glass slab and spatula being wiped with 70% ethanol between mixes. Each of the cements was mixed as per manufacturer instructions and dispensed into the appropriate wells in the agar, each of the cements being used to fill two of the four wells in each plate. The plates were then incubated for 24 hours at 37 degrees Celsius in a microaerophilic atmosphere .

2.2.2 ASSESSMENT OF THE ZONES OF INHIBITION

The presence or absence of zones of inhibition and the sizes of the zones were measured using callipers (Fig.2.4). The size of the zone was taken as the distance from the edge of the cement well to the periphery of the zone of inhibition. Each cement type was measured six times for each bacteria.

2.2.3 ASSESSMENT OF pH AT THE PERIPHERY OF THE ZONES OF INHIBITION

For each zone of inhibition, the pH at its periphery was measured with a palladium touch microelectrode (diameter 0.1mm, Beetrode MEPH-1; W.P. Instruments, New Haven, Conn.,USA) and a porous glass reference electrode (Beetrode MERE-1; W.P. Instruments). Both were connected to battery-run Orion SA 720 pH/ISE meters (ORION Research, Cambridge, Mass.,USA).

The examination comprised of the peripheral pH measurements for all the zone of inhibition on the twelve plates. Immediately prior to examining all the zones, the electrodes were calibrated against standard pH buffers at pH 4.00 and 7.00. If calibration curves showed a drift exceeding 0.05 pH units in any of the two buffers, the readings in between were adjusted according to calibration curves before the data analysis. The palladium touch microelectrode was inserted into the agar at the periphery of each zone of inhibition and the reference electrode was placed on the surface of the agar. On stabilising, a reading was taken and from the calibration curve

Figure 2.4: Typical zones of inhibition associated with Ultra Band-Lok after incubation for 24 hours at 37°C in microaerophilic conditions.

the pH was determined .

2.2.4 ASSESSMENT OF THE FLUORIDE GRADIENT FROM THE TEST CEMENTS IN UNINOCULATED PLATES

One 5mm diameter well was created in each of the six trypitic soy agar plates produced. One of the two cements was placed into the wells on two of the plates and the remaining two plates were left unfilled. The plates were incubated for 24 hours.

A small nylon punch was used to create 2mm diameter wells at 1mm, 5mm, and 10mm from each of the 5mm wells.The agar plugs were transferred to a microbalance (Cahn Instruments inc. Cerritos, California, USA) and weighed. The plugs were then transferred to a bijou tube.

To each bijou tube was added 0.5ml of deionised water and the agar dissolved over a water bath. 0.25 ml of each tube was transferred to a microsample dish and 0.25ml of low-level TISAB was added.

The ionic concentration of each solution was measured using an Orion combination fluoride ion-selective electrode (Orion Research Electrode No 9609BN) attached to an ion analyser (Orion Research Ion Analyser EA940, Boston Massachusetts, USA). For each of the plates a fluoride gradient was determined.

2.2.5 THE STATISTICAL ANALYSIS

The zones of inhibition and the pH at the periphery of the zones, of the two test cements for each of the test bacteria were compared using a Mann Whitney U test.

2.3 SPECIMEN PREPARATION AND MEASUREMENT OF MICROLEAKAGE OF BANDS CEMENTED WITH EACH CEMENT

2.3.1 SPECIMEN PREPARATION

Fifteen pairs of recently extracted, intact caries-free human third molar teeth without restorations were stored in 0.12 % thymol in individual labelled plastic containers until required. The teeth were hand scaled and cleaned to remove debris. Each tooth was rinsed under deionised water, the surface of the crown polished with pumice and water and then washed and lightly dried with cotton wool. For each tooth a prefabricated plain molar band (3M UNITEK, Bradford, England) was selected for best fit and adjusted to obtain good adaptation to the tooth contour.

Fifteen bands were cemented with Ketac-Cem and 15 bands were cemented with Ultra Band-Lok, one band in each tooth/band pair being cemented with each cement. Excess cement was removed from the occlusal surface using cotton wool rolls prior to cement set. The Ketac-Cem group was allowed to bench cure for 5 minutes and the Ultra Band-Lok group was light-cured from the occlusal surface for 60 seconds using an ESPE Elipar light (ESPE, Fabrik Pharmazeutischer Praparate, Seefeld, Germany).

After initial set the teeth were placed in a plastic storage vessel containing deionised water and were stored for 1 month at 37 degrees Celsius. The teeth were then removed from the deionised water and were lightly dried. Each tooth was painted with two coats of nail varnish to cover the root surface and the cervical aspect of the band and cement.

The teeth were allowed to dry by standing the teeth with the occlusal surface of the band on damp filter paper to prevent dehydration of the cement. After 15 minutes the teeth were artificially aged by thermocycling at 5, 37 and 55 degrees celsius for 850 cycles with a travel time of 30 seconds and a dwell time of 10 seconds according to the method of Scott (1994).

2.3.2 ASSESSMENT OF MICROLEAKAGE

2.3.2.1 Pilot study

In reviewing the literature, authors have used various times of immersion in 2% methylene blue dye to determine microleakage.

To assess the most acceptable time, three pairs of wisdom teeth were selected and a band was cemented to one tooth from each pair with one of the two test cements. The teeth were varnished and thermocycled using the method as described above. The teeth were immersed in 2.0% methylene blue dye buffered to pH 7 for either, 6 hours, 12 hours and 18 hours. On removal the teeth were sectioned and viewed under the light microscope to assess which dwell time in the dye gave the best visualisation of microleakage. Twelve hours in 2.0% metylene blue dye was found to give the most noticeable result.

2.3.2.2 Main study:

After thermocycling the teeth were dried lightly and immersed in 2.0% methylene blue buffered to pH 7 for 12 hours. On removal the teeth were lightly scrubbed with a nail brush under running water to remove excess dye and lightly dried. Each individual tooth was embedded in self-curing acrylic up to the occlusal surface of the band and sectioned longitudinally in a buccolingual direction through the mesiobucccal groove of the tooth, using a Microslice 2 hard tissue saw (Malvern Instruments, England) (Fig.2.5) (Fig.2.6).

A magnified (X10) photographic record with a standard background illumination showing the whole occlusal-cervical extent of the band and the extent of microleakage on the facial and lingual surface of each tooth was taken under a light microscope (Zeiss Stereomicroscope IV with a Nikon F3 camera) (Fig.2.7). This resulted in 60 photographs: one photograph of the buccal aspect and one of the lingual aspect of each of the thirty teeth.

Each photograph was numbered randomly from 1 to 60. The extent of microleakage was assessed independently by two examiners (a Senior Lecturer in Orthodontics, Dr D. Millett and a Senior Lecturer in Oral Sciences, Dr S. Creanor).

For each interface, enamel/cement and cement/band, microleakage was assessed and coded as follows:

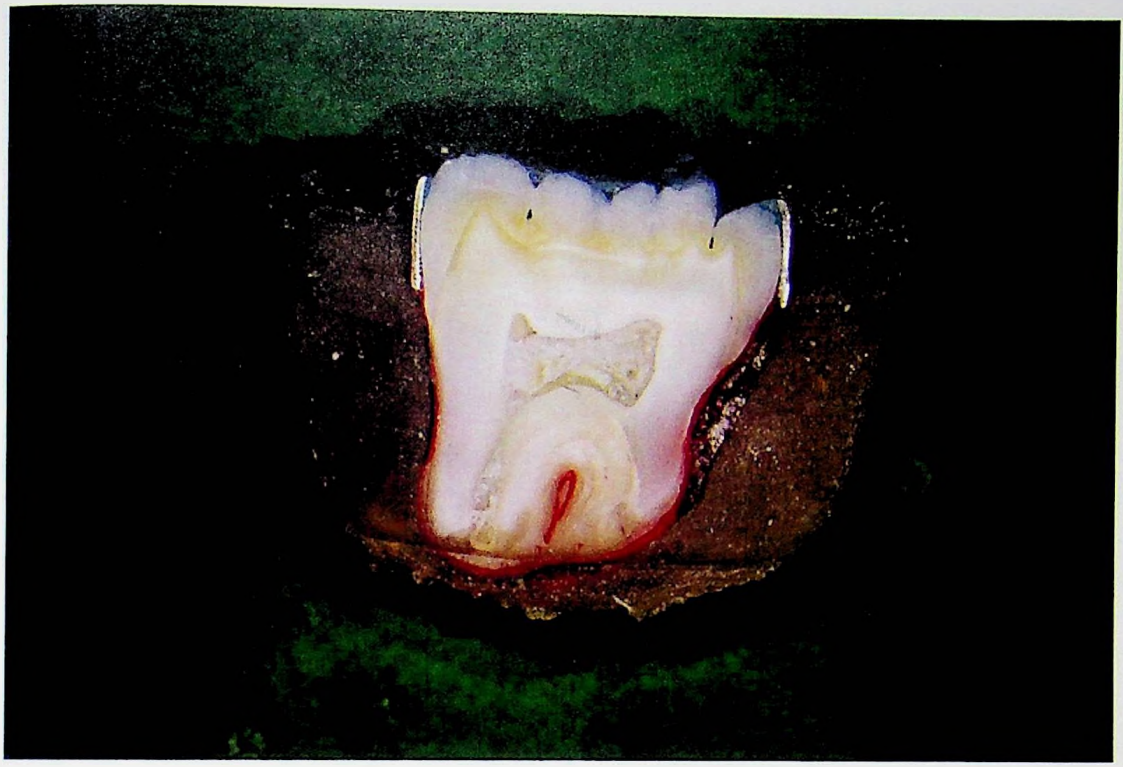

Figure 2.5: A banded wisdom tooth, embedded in self-curing acrylic up to the occlusal surface of the band and sectioned longitudinally in a buccolingual direction through the mesiobuccal groove.

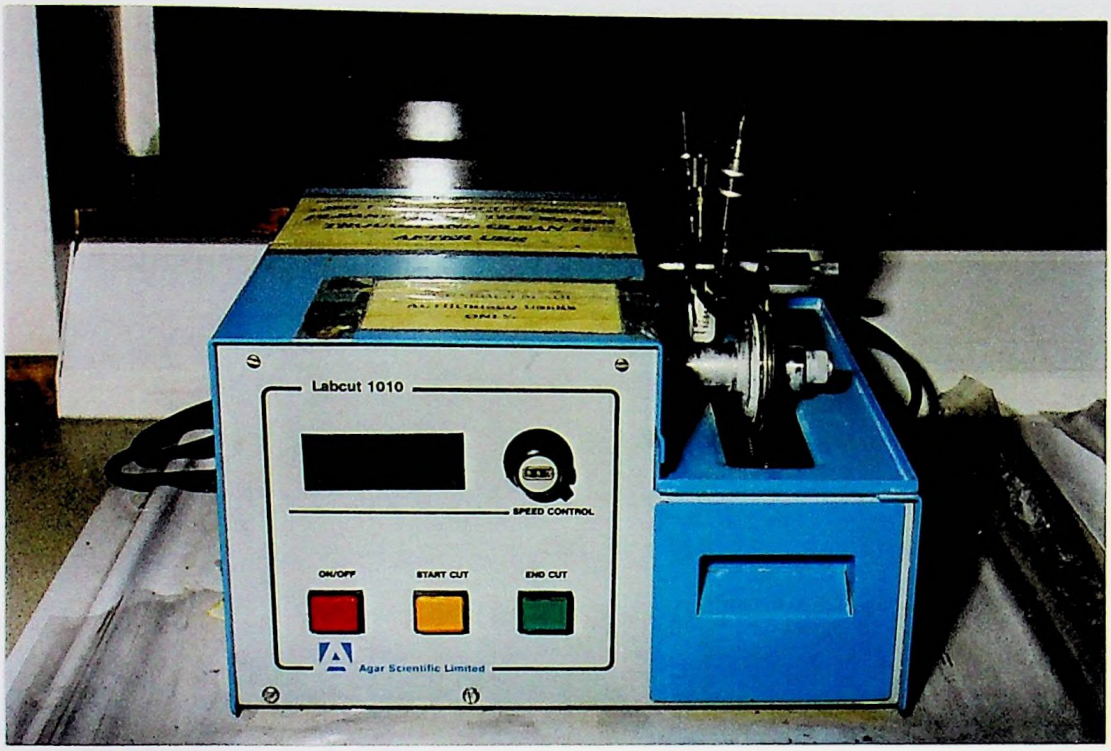

Figure 2.6: Microslice 2 hard tissue saw (Malvern Instruments, England).

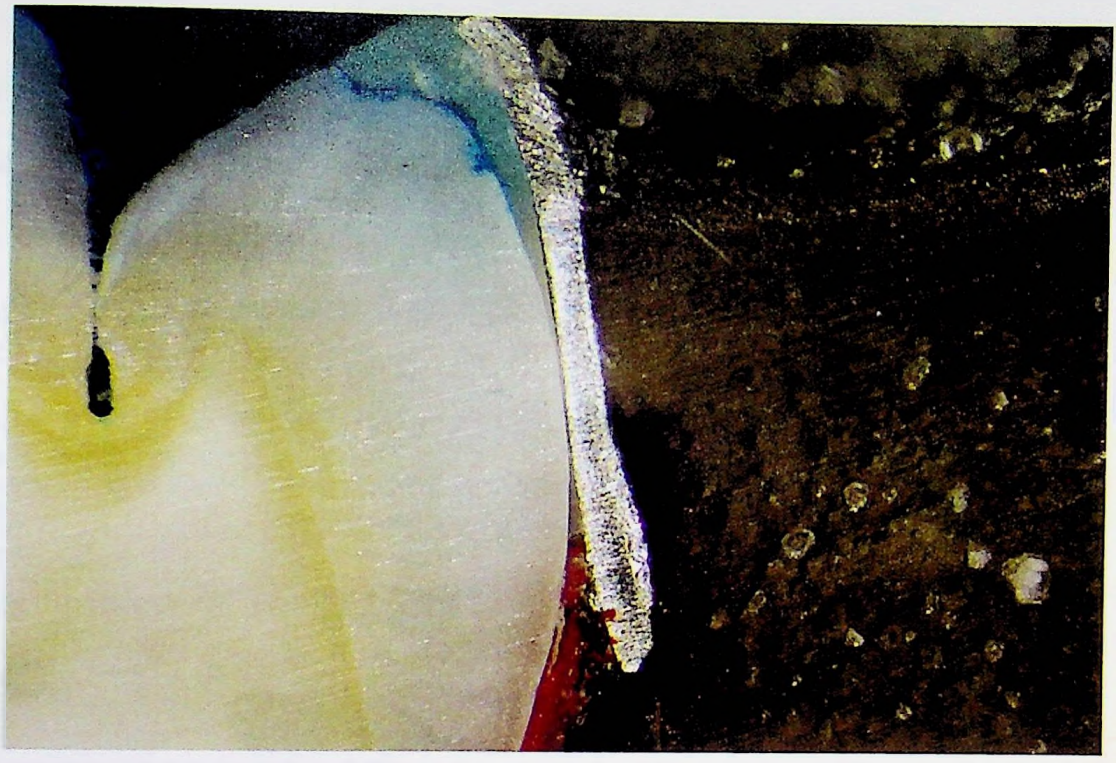

Figure 2.7: X10 magnified view of a sectioned banded wisdom tooth, showing microleakage at the cement/tooth interface.

Code 0 = no microleakage.

Code 1 = microleakage extends to <1/3 of interface.

Code 2 = microleakage extends to between 1/3 and 2/3 of the interface.

Code 3 = microleakage extends to >2/3 of the interface.

The presence/absence of cement surface loss (ditching) was also assessed.

Code 0 = ditching not present.

Code 1 = ditching present.

The examiners were unaware of which band had been cemented with each material. A random sample of 12 photographs was re-examined two weeks after the initial assessment to give an indication of inter- and intra-examiner reliability.

2.3.3 STATISTICAL ANALYSIS

Intra- and Inter- examiner reliability were assessed using a kappa statistic. The two test cements were compared at the cement/enamel and cement/band interfaces using a Mann Whitney U test.

CHAPTER 3

RESULTS

3.1 FLUORIDE RELEASE

3.1.1.COMPARISON OF THE PATTERN OF FLUORIDE RELEASE

The fluoride release pattern for each material was essentially the same (Fig. 3.1). The majority of the fluoride was released over the first 5 days, followed by a steady decline to day 15 when a plateau was reached. Thereafter, there was a slow reduction in the daily release to day 40.

3.1.2 COMPARISON OF THE DAILY MEAN AND CUMULATIVE TOTAL FLUORIDE RELEASE

The daily mean fluoride release and the cumulative mean fluoride release for Ketac-Cem and Ultra Band-Lok at Days 5, 10 and 40 days are given in Table 3.1 and Table 3.2 respectively. The results for days 5, 15 and 40 were compared for the test cements using a Mann Whitney U test. The results for both mean daily and mean cumulative fluoride release for days 5, 15 and 40 proved significant ($p < 0.05$).

Figure 3.1 Daily Fluoride Release from Ketac-Cem and Ultra Band-Lok

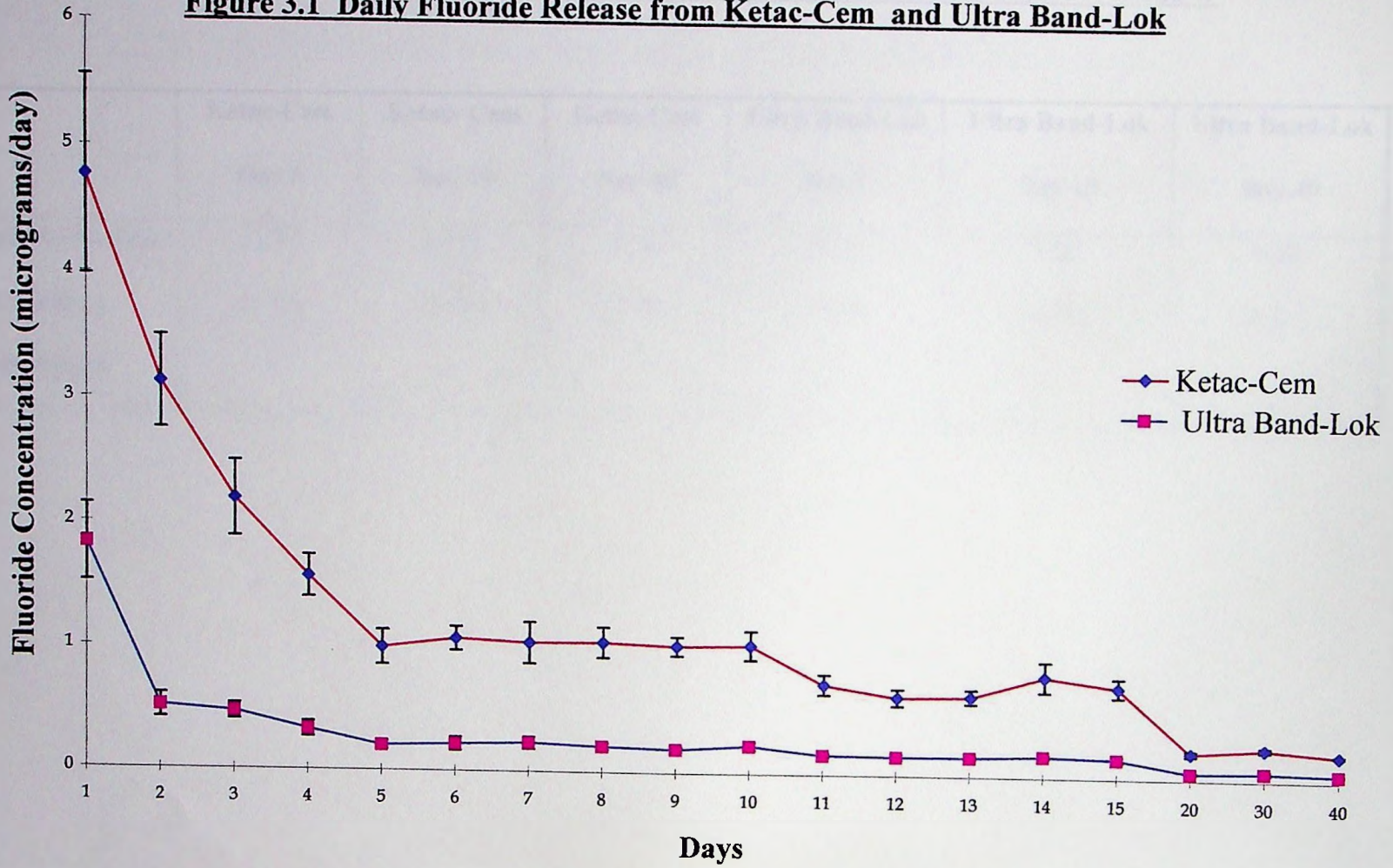

Table 3.2 Cumulative Mean Fluoride Release for Ketac-Cem and Ultra Band-Lok at days 5, 15 and 40

	Ketac-Cem Day 5	Ketac- Cem Day 15	Ketac-Cem Day 40	Ultra Band-Lok Day 5	Ultra Band-Lok Day 15	Ultra Band-Lok Day 40
Cumulative Release (Standard Deviation)	12.89 (1.33)	21.52 (2.00)	27.42 (2.25)	3.37 (0.50)	5.26 (0.60)	6.80 (0.69)

Table 3.1 Daily Mean Fluoride Release for Ketac-Cem and Ultra Band-Lok at days 5, 15 and 40

	Ketac-Cem Day 5	Ketac- Cem Day 15	Ketac-Cem Day 40	Ultra Band-Lok Day 5	Ultra Band-Lok Day 15	Ultra Band-Lok Day 40
Mean Daily Release (Standard Deviation)	1.01 (0.14)	0.74 (0.08)	0.21 (0.01)	0.20 (0.03)	0.16 (0.02)	0.06 (0.00)

3.2 ANTIMICROBIAL ACTIVITY

3.2.1 COMPARISON OF THE ZONES OF INHIBITION FOR THE TWO TEST CEMENTS

The mean zones of inhibition for the four Streptococcus mutans strains using Ketac-Cem and Ultra Band-Lok are given in Table 3.3 and shown in Figure 3.2.

The zones of inhibition for the two test cements for each bacterium were compared using a Mann Whitney U test. For each test bacterium, there was a significant difference between the two test cements ($p < 0.05$) with the antimicrobial activity of Ketac-Cem being greater than that of Ultra Band-Lok.

3.2.2 COMPARISON OF THE VALUES OF pH AT THE ZONES OF INHIBITION FOR EACH TEST CEMENT

Table 3.4 gives the mean value for pH at the periphery of the zone of inhibition and the standard deviation for each of the four Streptococcus mutans strains using Ketac-Cem and Ultra Band-Lok.

The statistical analysis was carried out using a Mann Whitney U test for each of the different bacteria, comparing the two test cements. There was no significant difference ($p > 0.05$) between the pH scores for A5 and A1, but a significant difference between the pH scores for C1 and A10 with the pH value for Ultra Band-Lok being the marginally higher value.

Figure 3.2 Zones of Inhibition for Ketac-Cem and Ultra Band-Lok for each Streptococcus mutans Strain

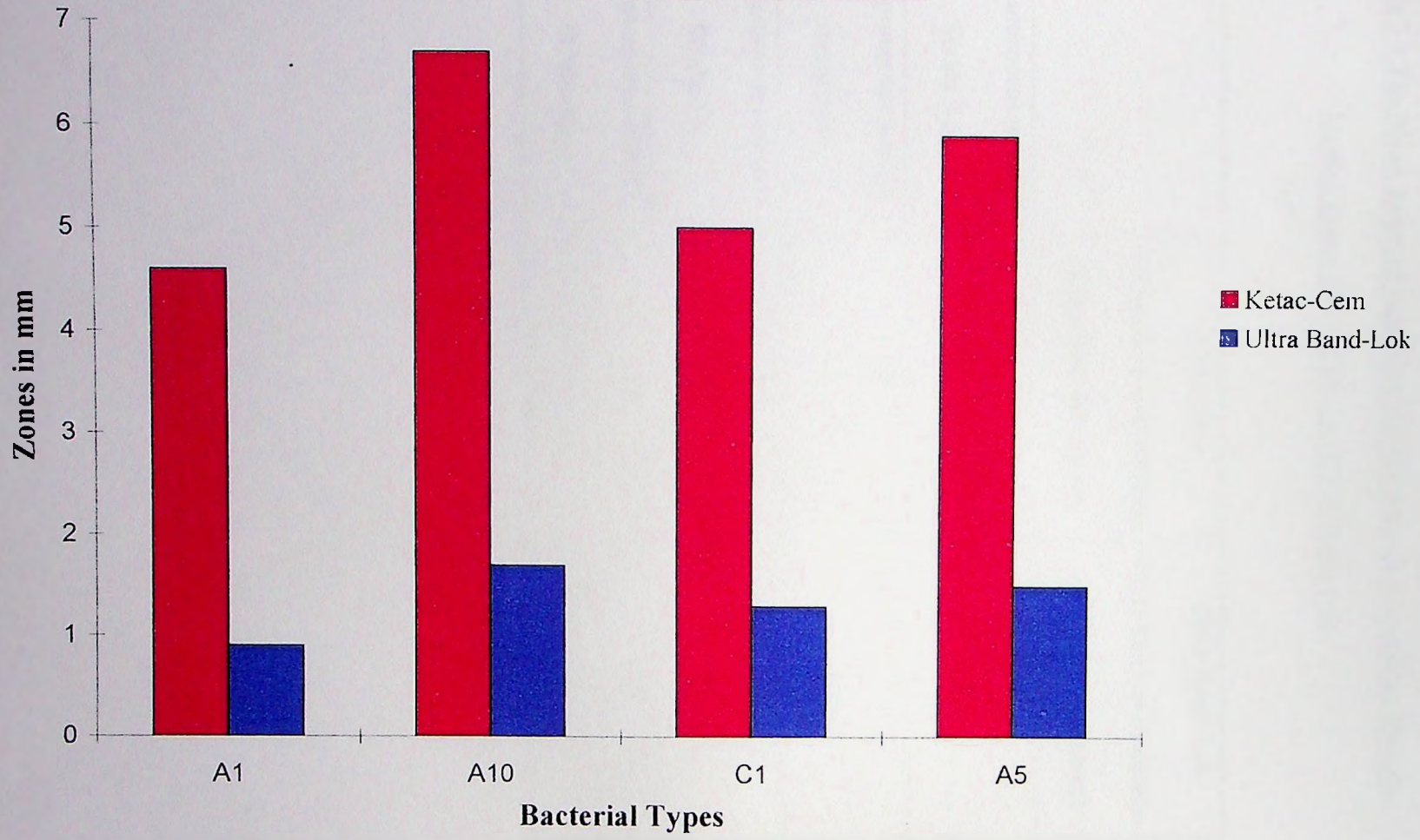

Table 3.3 The Mean Zones of Inhibition for the Four Streptococcus Mutans Strains Using Ketac-Cem and Ultra Band-Lok

	Ketac-Cem Mean Zone of Inhibition (Standard Deviation)	Ultra Band-Lok Mean Zone of Inhibition (Standard Deviation)
Streptococcus mutans Strain A1	4.6 (0.50)	0.9 (0.5)
Streptococcus mutans Strain A10	6.7 (0.4)	1.7 (0.2)
Streptococcus mutans Strain C1	5.0 (0.5)	1.3 (0.5)
Streptococcus mutans Strain A5	6.0 (0.5)	1.5 (0.3)

Table 3.4 The Mean pH Value at the Periphery of the Zones of Inhibition for the Four Streptococcus Mutans Strains Using Ketac-Cem and Ultra Band-Lok

	Ketac-Cem Mean pH value (Standard Deviation)	Ultra Band-Lok Mean pH value (Standard Deviation)
Streptococcus mutans Strain A1	5.13 (0.151)	5.23 (0.052)
Streptococcus mutans Strain A10	5.53 (0.051)	5.54 (0.048)
Streptococcus mutans Strain C1	5.25 (0.054)	5.41 (0.075)
Streptococcus mutans Strain A5	5.35 (0.055)	5.36 (0.051)

3.3 ASSESSMENT OF MICROLEAKAGE BETWEEN TWO TEST CEMENTS

3.3.1 INTRA- AND INTER-EXAMINER RELIABILITY

Intra- and inter-examiner kappa scores for cement/enamel and cement/band interfaces are given in Table 3.5. Most values are greater than 0.8 indicating a high level of agreement between the two examiners.

Two values for intra-examiner reliability were 0.71 representing an acceptable level of agreement. The reason for the relatively low scores was that although 20% of the photographs were re-examined, this only represents 12 photographs and as such one disagreement reduces the kappa score substantially .

3.3.2 RESULTS AT THE CEMENT/ENAMEL AND CEMENT/BAND INTERFACE

Table 3.6 and Figures 3.3 and 3.4 give the results in each category of microleakage at the cement/enamel and cement/band interfaces for Ketac-Cem and Ultra Band-Lok cements.

Table 3.7 gives the statistical analysis using a Mann Whitney U test for the cement/enamel and cement/band interfaces between the two test cements for each examiner and the mean result for the two examiners. Using a Mann-Whitney U test to test for significance at the cement/enamel junction the result gave a p value of 0.66

Table 3.5 Kappa Values for Intra- and Inter-Examiner Reliabiltities Assessed by Microleakage at the Cement/Enamel and Cement/Band Interfaces

	Cement/Enamel Examiner 1	Cement/Enamel Examiner 2	Cement/Band Examiner 1	Cement/Band Examiner 2
Examiner 1	0.71	0.92	0.87	0.84
Examiner 2	0.92	0.71	0.84	0.87

Table 3.6 Microleakage Scores at Cement/Enamel and Cement/Band Interfaces for Ketac-Cem and Ultra Band-Lok

	Cement/Enamel Microleakage Score 1	Cement/Enamel Microleakage Score 2	Cement/Enamel Microleakage Score 3	Cement/Band Microleakage Score 2	Cement/Band Microleakage Score 2	Cement/Band Microleakage Score 3
Ketac-Cem	8	8	14	9	11	10
Ultra Band-Lok	6	10	14	5	8	17
Total	14	18	28	14	19	27

Figure 3.3 Microleakage at the Cement/Enamel Interface for Ketac-Cem and Ultra Band-Lok

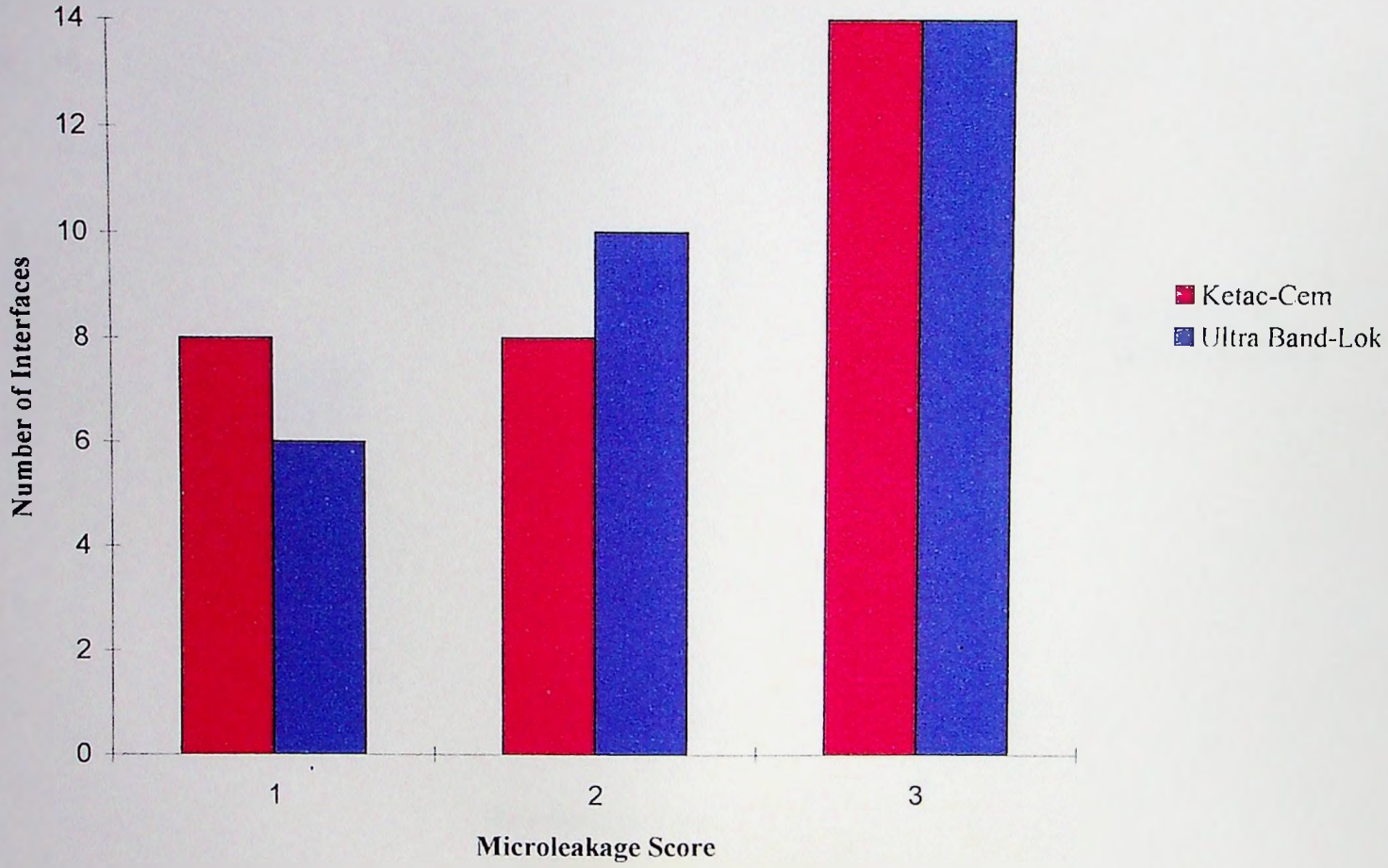

Figure 3.4 Microleakage at the Cement/Band Interface for Ketac-Cem and Ultra Band-Lok

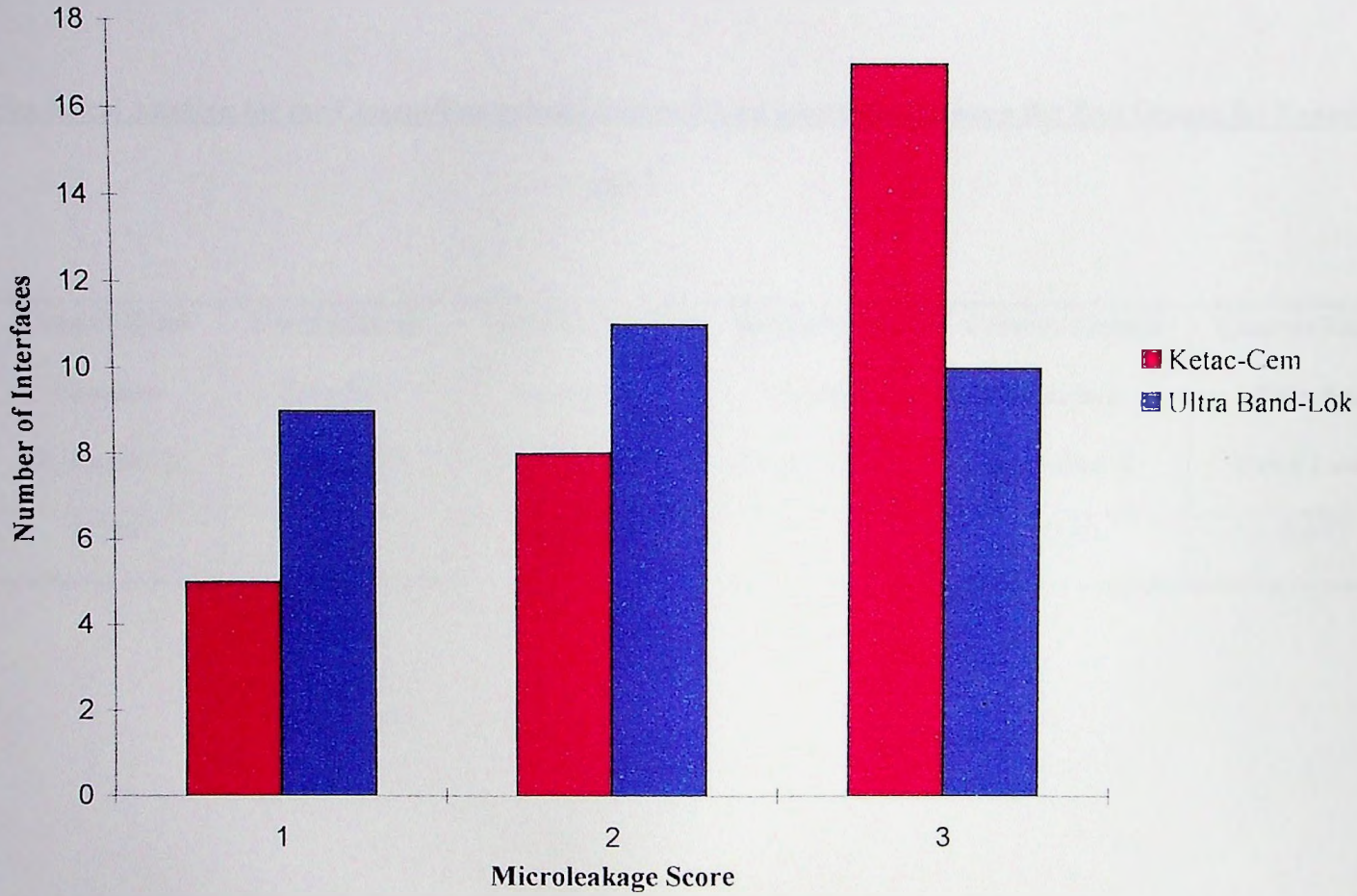

Table 3.7 The Statistical Analysis for the Cement/Enamel and Cement/Band Interfaces Between the Two Groups for Examiners 1 and 2

	Cement/Band Interface Examiner 1	Cement/Band Interface Examiner 2	Cement/Band Interface Mean 1 and 2	Cement/Enamel Interface Examiner 1	Cement/Enamel Interface Examiner 2	Cement/Enamel Interface Mean 1 and 2
p Statistic	0.059	0.039	0.051	0.665	0.575	0.657

indicating no significant difference for the two test cements.

Using a Mann-Whitney U test to test for significance at the cement/band junction the result gave $p = 0.051$ suggesting a result of borderline significance for the two test cements.

CHAPTER 4

DISCUSSION

To reduce the incidence of decalcification on orthodontically banded teeth, a band cement should ideally release fluoride over the duration which the tooth is banded, possess antimicrobial activity capable of inhibiting cariogenic bacteria and prevent microleakage between the cement and enamel interface. The results of each of these aspects undertaken in the present study will now be discussed.

4.1 FLUORIDE RELEASE

4.1.1 VALIDITY OF THE STUDY DESIGN AND METHODS OF FLUORIDE ASSESSMENT.

Table 1.3 outlines previous orthodontic studies using a similar method of fluoride assessment to that used in the present study.

4.1.1.1 The Potentiometric Method of Fluoride Detecton.

Cranfield (1982) first developed the method of detection of a fluoride ion in vitro by which an ion specific electrode is connected to an ion specific meter. Since its introduction it has become a widely recognised and used method of assessing fluoride

concentration in solution. Of the 11 studies listed under the disc model in Table 1.3 all have used the potentiometric method for assessment of fluoride release.

4.1.1.2 Pellets

There appears to be no consensus on the size or shape of pellets to be used for assessing fluoride release. Previous orthodontic studies have used disc-shaped cylinders (Swartz, 1984; Chan, 1990; Ogaard et al., 1992; Newman and Randolph, 1994; Wiltshire and Janse Van Rensberg, 1995; Monteith 1997) rectangles or squares of material (Fox, 1990; Chadwick and Gordon, 1995). Specimen size has also varied between studies from 3mm x 1.5mm (Monteith, 1997) to 30mm x 20mm x 2mm rectangle (Lynch and Tay, 1989). Yip (1995) used two different sample dimensions when he assessed the fluoride release from light cured hybrid glass-ionomer cements and found the smaller dimension of the pellets had the more consistent release.

It would prove extremely difficult to produce a pellet small enough to have an equivalent volume to that of the cement associated with a banded tooth. Therefore, dimensions of 3mm by 1.5mm were chosen as these were the smallest that could easily be manufactured and handled and are the same as used by Monteith (1997).

4.1.1.3 Medium

Previous work has shown fluoride to be released more readily into deionised water than into artificial saliva (Saito, 1978; Mallakh and Sarkar, 1990) though the situation with artificial salivas may not necessarily be clinically relevant. Many workers have used a

pellet and deionised water to assess fluoride release from orthodontic cements (Swartz, 1984; Cooley et al, 1989; Lynch and Tay, 1989; Fox, 1990; Chan et al., 1990; Ogaard et al., 1992; Newman and Randolph, 1994; Chadwick and Gordon, 1995; Monteith, 1997). The volume of deionised water chosen in the present study was 2ml. The volume used previously for orthodontic studies varies between 2ml (Monteith, 1997) to 30ml (Newman and Randolph, 1994). Two millilitres was chosen for this study as small pellets were used and each could be completely covered by this volume in the tube.

4.1.1.4 Sample Size

Five disc specimens of each material were prepared in this study. Although previous studies have used various numbers of specimens in each group from two (Wyness and Sheriff, 1996) to twenty (Chan et al., 1990), five disc specimens in each group of test cements have been used by Cooley et al. (1990) and Chadwick and Gordon (1995). From the results it is clear that there was consistency between samples and therefore, this sample size allowed a clear difference to be detected between the two test groups for fluoride release.

4.1.1.5 Observation Time

From previous studies using glass-ionomer cement, most of the fluoride release occurs over the first 5 days though observation times have been substantially longer; 1 year, Swartz (1984); 1.5 years Newman and Randolph (1994) and 85 months Wiltshire and Janse Van Rensberg (1995).

Previous studies using Ketac-Cem and a disc model have shown a rapid release over the first 7 days followed by a steady fall in the release of fluoride by the pellet, reaching a plateau by 2 weeks (Swartz, 1984; Fox, 1990). The present study has an observation time of 40 days. The relevance of taking readings beyond this time period, with the low levels of fluoride likely to be present, is questionable.

4.1.1.6 Units

Previous studies have used μg/cm^2 (Chan, 1990; Wiltshire and Janse Van Rensberg, 1994) others have used μg/day (Swartz 1984; Fox, 1990) or ppm (Ogaard, 1992; Ghani, et al., 1994; Monteith, 1997). The units in this study are μg/day but these may be easily converted to ppm or μg/cm^2 (area 0.28cm^2 for a disc 3mm diam x 1.5mm height).

4.1.2 FLUORIDE RELEASE RESULTS:

4.1.2.1 Fluoride Release from the Pellets

The pattern of fluoride release from the pellets of Ketac-Cem is consistent with that of other studies using this cement (Swartz, 1984; Fox, 1990).

The fluoride released from the Ultra Band-Lok follows a similar pattern to that of Ketac-Cem though releasing a smaller amount of fluoride at each time interval. The pattern of fluoride release matches that described by Ashcraft et al. (1997) for Band-Lok and is similar to other hybrid glass-ionomer cements (Mitra, 1991; Creanor et al.,

1994; Monteith, 1997).

The pellets of Ketac-Cem released more fluoride than those of Ultra Band-Lok and this result proved statistically significant for 5 days, 14 days and 40 days.

In comparing this study with other in vitro studies where the fluoride release from pellets of Ketac-Cem has been measured, the results obtained in this study are substantially smaller. Swartz (1984) reported a total mean release of fluoride in the first 2 days to be 150μg, while Fox (1990) found a release of 180μg over 2 days. Although the present study only found a release in the first two days of 8.64μg, it is important to note that these studies are not directly comparable as the pellets used by Swartz (1984) and Fox (1990) were much larger than those used in the present study.

A more useful comparison for fluoride release is from the work of Monteith (1997) who used pellets of the same size to those used in the study reported here. Her results, although not using either Ketac-Cem or Ultra Band-Lok did assess two hybrid glass-ionomer cements and their fluoride release falls within a similar range of values to those found in the present study. She found that Vitremer released 4.78μg in the first day and Dyract 1.31μg, in comparison to the present study where Ketac-Cem released 5.51μg and Ultra Band Lok 2.34μg.

Only one previous study has compared Ketac-Cem directly with a hybrid glass-ionomer cements for fluoride release as in the present study (Wyness and Sheriff, 1996). They found that the fluoride release from Ketac-Cem was greater than from Dyract and another hybrid glass-ionomer cement (EXM#169). The fluoride release from Ketac-Cem in the present experiment also appears greater than that released from Dyract in

the study by Monteith (1997). This result is particularly relevant to the present study as the chemical nature of Dyract appears similar to that of Ultra Band-Lok.

Only Ashcraft et al. (1997) have investigated the fluoride release from Band-Lok (a two paste preparation rather than the one paste preparation, Ultra Band-Lok). They assessed the fluoride release of three light-cured, hybrid glass-ionomer cements and one chemically-cured composite resin cement using a tooth-bracket model. They found that Band-Lok released more fluoride than the other hybrid glass-ionomer cements (Zionomer and Geristore) and was superior to composite resin (Concise). The reason for these results they explained was due to Band-Lok having a chemistry more akin to conventional glass-ionomer cements than the other test materials. In general terms it appears the more similar the chemistry of the hybrid glass-ionomer is to a conventional glass-ionomer cement the greater their fluoride release (Ashcraft et al, 1997; Monteith, 1997).

The results of this study indicate that the fluoride release from Ultra Band-Lok is at least comparable to that of other hybrid glass-ionomer cements. This is interesting as Gasser (1994) has suggested that fluoride release is likely to be very low from hybrid glass-ionomer cements without a glass/acid reaction.

4.2 ANTIMICROBIAL ACTIVITY OF TEST CEMENTS

4.2.1. VALIDITY OF THE STUDY DESIGN AND METHODS OF ANTIMICROBIAL ACTIVITY ASSESSMENT:

Table 1.5 reviews the in vitro methods of assessing antimicrobial activity and indicates previous orthodontic studies where these methods have been used.

4.2.1.1 Agar Diffusion Test

As previously stated the agar diffusion test is the most common method of assessing antimicrobial properties of dental materials (Tobias 1988), although only Pavic et al. (1990) have used this method to assess orthodontic cements. There are two basic methods; where a disc of set material is placed on a previously inoculated plate (Pavic et al., 1990; Fischmann and Tinanoff, 1994; Palenic and Setcos 1996) or where a well is created in inoculated agar and freshly mixed unset material is placed in the well and set (McComb and Ericson, 1987; Barkhordar et al., 1989; DeSchepper et al., 1989a,b). Larger zones of inhibition are created by the latter method. Zones of inhibition have been found for both conventional glass-ionomer cement (McComb and Ericson, 1987; DeSchepper et al., 1989a,b) and hybrid glass-ionomer cements (DeSchepper et al., 1989b) when the plates are incubated with Streptococcus mutans.

4.2.1.2 Size of the Test Group:

The numbers of test specimens vary between studies with the range being two wells per cement group (Barkhordar et al 1989) to five wells per cement group (DeSchepper et al. 1989a). In both of these studies significant differences were found between groups. The present study used six wells per test cement for each different bacterial type as this was felt sufficient to demonstrate significance between the test cements.

4.2.1.3 Time of Incubation

DeSchepper et al. (1989a,b) incubated his inoculated plates overnight, while McComb and Ericson (1987) and Palenik and Setcos (1994) chose 48 hours. Pavic et al. (1990) adopted an incubation time of 72 hours and Barkholder et al. (1989) chose 7 days incubation. In the present study an incubation time of 24 hours was chosen as a previous study has found, using the agar diffusion test, that after 24 hours there is a substantial reduction in the antibacterial properties of glass-ionomer cements (Tobias et al. 1985). 24 hours also gave the most easily identified zones of inhibition without allowing overgrowth of the test bacterium.

4.2.1.4 Type of Bacterium

The most commonly employed test bacterium when using the agar diffusion test for dental materials is Streptococcus mutans (McComb and Ericson, 1987; Barkhordar et al., 1989; DeSchepper et al., 1989a,b; Pavic et al., 1990; Palenik and Setcos, 1994;

Fischmann and Tinanoff 1996). Others have been used; Lactobacillus Casei (McComb and Ericson, 1987; Pavic et al., 1990; Palenik and Setcos, 1994); Streptococcus Sanguis (Barkhordar et al 1989; Palenik and Setcos, 1994; Fischman and Tinanoff, 1996), Streptococcus Sobrinus (Palenik and Setcos, 1994; Fischman and Tinanoff, 1996) Actinomyces Viscosus (Palenik and Setcos, 1994; Fischman and Tinanoff, 1996); Staphlococcus aureus (Palenik and Setcos, 1994); Pseudomonas aeruginosa (Palenik and Setcos, 1994); Candida albicans (Palenik and Setcos, 1994) and Salmonellas choleraesuis (Palenik and Setcos, 1994).

Streptococcus mutans infection has been strongly associated with the development of dental carious lesions (Hamada and Slade, 1980) and the levels of Streptococcus mutans have been found to increase in patients wearing fixed orthodontic appliances (Corbett et al., 1981; Lundstrom and Krasse, 1987). The present study assessed the antimicrobial activity of the two test cements against Streptococcus mutans as any reduction in the activity and numbers of these bacteria associated with orthodontic bands may have the benefit of leading to a reduction in the likelihood of demineralisation associated with banded teeth.

4.2.2 ANTIMICROBIAL ACTIVITY RESULTS

4.2.2.1 Zones of Microbial Inhibition Relating to Ketac-Cem

The zones of microbial inhibition relating to Ketac-Cem fell within a similar range to those found in previous studies using conventional glass-ionomer cement, a similar methodology and Streptococcus mutans (McComb and Ericson, 1987; DeSchepper et al., 1989a). McComb and Ericson (1987) used a conventional glass-ionomer lining cement and found zones of inhibition of 4.5 mm when the plates were incubated for 48 hours. Barkhordar et al. (1989) found a mean inhibition zone for Ketac Bond of 3.2mm after 7 days. DeSchepper et al. (1989a) used Ketac-Cem as one of his test glass-ionomer cements and found zones of inhibition of 2.4mm after incubating the plates overnight. The present study found mean zones of inhibition for Ketac-Cem ranging from 4.7mm to 6.6mm.

4.2.2.2 Zones of Microbial Inhibition Relating to Ultra Band-Lok

Although no previous studies have used an agar diffusion test to assess Ultra Band-Lok for antimicrobial activity, the zones of inhibition found are in a similar scale to those found for other hybrid glass-ionomer cements. DeSchepper et al. (1989b) assessed the antibacterial properties of light-cured hybrid glass-ionomer liners and found that although not all materials displayed antibacterial properties two (XR Ionomer and Vitrabond) produced zones of inhibition between 3.3-5mm after incubation overnight

The present study found zones of inhibition for Ultra Band-Lok of between 0.3-1.7mm.

4.2.2.3 Comparison of the Zones of inhibition

The wells containing Ketac-Cem produced significantly more inhibition of all four types of Streptococcus mutans than the wells containing Ultra Band-Lok. These results do not support the conclusions of DeSchepper et al. (1989b) that the antibacterial activity of light-cured hybrid glass-ionomer cements is similar to that of conventional glass-ionomer cements.

McComb and Ericson (1987) suggest that the antibacterial effect of glass-ionomer cements may be due to either fluoride release or to the drop in pH as the cements set, this has been supported by DeSchepper et al. (1989a). Fischman and Tinanoff (1994) found a consistent relationship between the depression of pH but no relationship between the amount of fluoride released from the glass-ionomer cements with the presence and size of the zones of inhibition. Loyola-Rodriguez et al. (1994) suggest that the effect of pH on the antimicrobial activity of glass-ionomer cements is only present when the cements are setting and that after setting the antimicrobial activity is due to their fluoride release. This confirms the work of previous studies (Berg et al., 1990; Forss et al., 1991). The diffusion of fluoride into agar from a set cement is likely to be slow and limited. Ultra Band-Lok which was snap-set by light-curing would therefore have little pH effects and its antimicrobial activity would be due to the limited amounts of fluoride released into the agar. The resulting zones of inhibition for Ultra Band-Lok therefore, would be quite small compared to those of Ketac-Cem where the

set is chemical over a period of 24 hours, allowing antimicrobial activity form both a lowered pH and fluoride release.

From the present study it is interesting to note that the pH at the zones of inhibition was relatively consistent, ranging between 5.1 to 5.8. These values are higher than those reported to be bactericidal by themselves (pH4) for Streptococcus mutans (Davies, 1967), and higher than those found by DeSchepper et al. (1989a) within the inhibition zones (pH3) suggesting that the pH is not the only source of antimicrobial activity.

The fluoride concentration ranging from 34-47ppm was found in the agar at 5mm from the Ketac-Cem well (zones of inhibition for Ketac-Cem range 4.7-6.6mm). This concentration of fluoride is within the range of the minimum concentration thought necessary to inhibit the growth of Streptococcus mutans 20-300ppm (Hamilton and Bowden, 1988) particularly given the reduced pH in the region.

At 1mm from the Ultra Band-Lok wells (zones of inhibition for Ultra Band-Lok range 0.3-1.7mm) the agar was found to have a concentration of fluoride of 5.5-6.7ppm. Although this figure is slightly outside the range quoted for antimicrobial activity with Streptococcus mutans the pH value was also reduced in the region increasing the sensitivity of the bacteria to fluoride.

These results, in combination, appear to agree with those of McComb and Ericson (1987) and DeSchepper et al. (1989a) who suggest that the antimicrobial activity of glass-ionomer cement is due to a combination of fluoride releaase from the cements and a reduced pH during the setting reaction which leads to an increased sensitivity of the bacteria to fluoride.

4.3 MICROLEAKAGE Of THE TEST CEMENTS

4.3.1 VALIDITY OF THE STUDY DESIGN AND METHODS OF MICROLEAKAGE ASSESSMENT.

Table 1.7 summarises the advantages and disadvantages of methods in assessing microleakage. The method used to compare the microleakage of bands cemented with the two test cements was the dye penetration method which has been discussed in section 1.4.

4.3.1.1 Dye Penetration Method.

It has been suggested that the dye penetration method is the most popular technique for assessing microleakage of dental cements (Taylor and Lynch, 1992) as it is simple, inexpensive and does not require exposure to hazardous chemicals (Going, 1985). Two percent methylene blue appears to be the most commonly used dye for dye penetration tests (Delvianis and Chapman, 1983; Scott, 1994; Pupalla et al., 1996) although other concentrations have been used: 0.5% (Ovrebo and Raadal, 1989) and 0.05% (Um and Oilo, 1992) amongst others.

The time of immersion in methylene blue dye has varied between 25 minutes (Ovrebo and Raadal, 1990) and 1 week (Greene, 1990). The time of immersion chosen for the present study was 12 hours which was found in a pilot study to give the most favourable results for analysis.

4.3.1.2 The Number in Each Test Group

Previous studies comparing different luting cements for stainless steel crowns have used five teeth per test cement (Berg et al., 1988; Shiflett and White, 1997). Studies using restorative cements vary between eight teeth per test group (Pupalla et al., 1996) and twenty teeth per test group (Reid et al., 1994; Saunders and Saunders, 1996). Shiflett and White (1997) found that groups of just five per test cement were too small to determine a difference between the adhesive cement groups. Hallet and Garcia-Godoy (1993) found a significant difference between conventional and hybrid glass-ionomer cements using 15 teeth per group. The present study used 15 teeth per test cement group.

4.3.1.3 Storage Time

Storage time prior to immersion in the dye has been shown to increase microleakage using restorative materials (Lim et al., 1989; Crim and Garcia-Godoy, 1987) although others have suggested that after the third day in assessing glass-ionomer luting cements there is a stabilisation of microleakage (Berg et al., 1987). The present study used a storage time of 1 month.

4.3.1.4 Thermocycling

Thermocycling has a widespread role in demonstration of marginal adaptation and microleakage (Taylor and Lynch, 1992). Crim and Garcia-Godoy (1987) found that thermocycling even for a short-term has a significant impact on microleakage and suggested that investigations incorporating thermal changes are more potent at demonstrating leakage than non-cycled methods Previous studies have used varying times of immersion in the water baths and varying temperature ranges for thermocycling. The cycles involved range between 500 (Scott, 1994) to 2000 (Shiflett and White, 1997) although Crim and Garcia-Godoy (1987) have concluded that cycling durations have little impact on microleakage patterns. The present study used 850 cycles.

The dwell times in the thermal baths used by previous studies has also varied, usually between 10 seconds as used by Scott (1994) and 30 seconds used by Shiflett and White (1997). Scott (1994) advised that the dwell times should be short to resemble the oral environment, as previous work has found that teeth come into contact with temperature extremes for only short periods in the mouth (Longman and Pearson, 1987). A 10 second dwell time was adopted for this study for that reason.

4.3.1.5 The Index

An index rather than linear measurement is frequently used in dye penetration studies for restorative materials (Crim and Mattingly, 1981; Crim and Garcia-Godoy, 1987; Ovrebo and Radaal, 1989; Hallet and Garcia-Godoy, 1993; Reid et al., 1994; Puppala et al., 1996; Saunders and Saunders, 1996). No previous studies on microleakage have been conducted using orthodontic bands although the index has similarities to that used by Saunders and Saunders (1996) who assessed the microleakage in restorative cements using 2% methylene blue. The scoring of the depth of dye penetration was according to the following criteria: 0= no leakage; 1= dye penetration less than one-third the cavity depth; 2= dye penetration to two-third the cavity depth; 3=dye penetration to the full depth of the cavity.

4.3.2 INTRA- AND INTER-EXAMINER RELIABILITY

A measure of the intra- and inter-reliability was determined using the Kappa statistic. The intra-examiner reliability was determined by re-examining 20% of the original sample (12 photographs) after a two week interval and using the kappa statistic to give a measure of reliability. The kappa statistic for intra-examiner reliability was acceptably

high with all the values being k >0.7. The value of k 0.71, although appearing relatively low is due to the small number of re-examined photographs and merely represents variations in two of the recordings between each of the examinations. The inter-examiner reliability for the cement/band interface was k 0.84 and for the cement/enamel was k 0.92, both acceptably high.

4.3.3 COMPARISON OF THE TWO CEMENTS:

When examining the results of this study it is important to note that we are only comparing the results from one point of sectioning, resulting in two areas of observation; one buccal and one lingual. The assumption, therefore, must be that these sites are representative of the whole tooth (Gale et al., 1994). In the present study more than one recording for each sectioned tooth was taken as advocated by Dejou et al. (1996). This ammounted to one recording from the lingual interface and one recording from the buccal interface for each sectioned tooth.

There are a number of difficulties in comparing the results of this study with those of other workers. No previous microleakage studies have been carried out on banded teeth and as such the nearest comparison is with that of studies using stainless steel crowns (Berg et al., 1988; Shiflett et al., 1997). The results derived from this study are also difficult to compare directly with others due to different methods of assessing

microleakage, criteria chosen for assessment and the statistical analyses (Dejou et al., 1996).

4.3.3.1 Cement/Enamel Interface

Average values between the two examiners have been used to assess the two interfaces as both examiners have a similar kappa score and are therefore equally reliable. Neither of the glass-ionomer cements examined in this investigation completely resisted microleakage at the enamel/cement interface. This result is similar to those of other investigators who have assessed restorative cements (Tseunekawa et al., 1992; Hallet and Garcia-Godoy, 1993) and luting cements (Berg et al., 1988; Shiflett and White, 1997).

Both of the tested cements in this study give a similar pattern of groupings in the three categories at the cement/enamel interface, with no significant difference between the two test cements (p 0.66). Hallet and Garcia-Godoy (1993) found variable microleakage against enamel when using two types of light-cured hybrid glass-ionomer cement restorations and two conventional glass-ionomer cement restorations. Shiflett and White (1997) were unable to find a significant difference between hybrid and conventional glass-ionomer cements for cementing stainless steel crowns on primary molars, but did find that they were capable of reducing microleakage compared with that of non-adhesive cements. Band-Lok has been found to behave more like a composite resin than a glass-ionomer cement. Phijaisant and Tyas (1997) have shown that Band-Lok has acceptable inherent strength but has inadequate bond strength to

enamel. It has been suggested that the poor bond strength to enamel is due to Band-Lok not containing an acid-polymer. The main means of retention for Band-Lok to enamel appears to be mechanical as with composite resin.

If we presume that as with Band-Lok, Ultra Band-Lok has poor chemical adhesion to enamel it is interesting that there was no significant difference between Ketac-Cem and Ultra Band-Lok for microleakage at the cement/enamel interface particularly as with other studies comparing adhesive and non-adhesive cements have found that those cements that adhere to enamel reduce microleakage (Shiflett and White, 1997).

This result may be due to hybrid glass-ionomers undergoing an initial greater setting contraction than conventional glass-ionomer cements due to polymerisation of the resin component (Hinoura et al., 1992), but unlike conventional glass-ionomer cements, they undergo hygroscopic expansion of up to 6% at 24 hours (Irie et al., 1992). This mechanism has been used to explain the lack of microleakage with restorative hybrid glass-ionomer cements (Crim, 1993).

4.3.3.2 Cement/Band Interface

As with the cement/enamel interface, average values were used to compare the two test cements. These suggested that Ketac-Cem was more prone to microleakage on the cement/band interface than Ultra Band-Lok, this result was found to have a borderline significance ($p=0.051$). From this if the assumption is made that greater microleakage

is likely to correlate with a higher failure rate at this interface, then this result would agree with the in vitro study of Millett et al. (1996) and the in vivo study of Fricker (1997). Both studies found, as previously stated, that when comparing Ketac-Cem with Band-Lok, although there was a largely mixed mode of failure, Ketac-Cem was more likely to fail at the cement/band interface than Band-Lok. Previous studies have shown that the adhesion of glass-ionomer cement to enamel is greater than to stainless steel (Maijer and Smith, 1988; Fricker, 1990). This has been used to explain why a band cemented with conventional glass-ionomer cement is more likely to fail at the cement/band interface than at the cement/enamel interface (Fricker, 1997).

When assessing in vitro microleakage studies, there are limited comparisons with what can be seen clinically (Roulet et al., 1994). It has been suggested that in vitro results should be considered as the maximum theoretical value of microleakage (Pashley, 1990). It is difficult to determine what would be an acceptable depth of microleakage, as its consequence for example demineralisation, probably relates more to the volume of leakage rather than to the depth of leakage (Roulet et al., 1994). Volume of leakage was not assessed in the present study.

The results of the two previous parts of this study have found that Ketac-Cem releases significantly more fluoride and has a significantly greater antibacterial activity over the first 24 hours than Ultra Band-Lok. These results should also be taken into account when considering the consequences of the microleakage experiment although Band-Lok has been shown to absorb extrinsic fluoride and this may give it some increased

cariostatic potential (Ashcraft et al., 1997). However in vitro demineralisation studies and clinical trials would be required to investigate this further.

CHAPTER 5

CONCLUSIONS AND SUGGESTIONS FOR FURTHER WORK

5.1 CONCLUSIONS:

The aims of this thesis were outlined in section 1.5. The conclusions relating to each are given here.

AIM 1: To compare the fluoride releasing characteristics of two orthodontic band cements, a light-cured hybrid glass-ionomer cement (Ultra Band-Lok) and a conventional glass ionomer (Ketac-Cem)

CONCLUSION: The daily and cumulative fluoride release from Ketac-Cem is significantly greater than that from Ultra Band-Lok on days 5, 15 and 40 ($p < 0.05$).

AIM 2: To compare the antimicrobial activity of the two cements against four strains of Streptococcus mutans using an agar diffusion test.

CONCLUSION: The antimicrobial activity of Ketac-Cem is significantly greater than that of Ultra Band-Lok against all the four strains of Streptococcus mutans tested ($p < 0.05$).

AIM 3: To compare the position and extent of microleakage when the two materials are used to cement orthodontic bands to third molar teeth.

CONCLUSION: There appears no significant difference in microleakage between Ketac-Cem and Ultra Band-Lok at the cement/enamel interface when used to cement orthodontic bands to third molar teeth in vitro ($p > 0.05$). At the cement/band interface, however, there is a suggestion of a difference between the two groups.

This thesis addressed the following **null hypotheses** as outlined in chapter 1 (section 1.5).

1. There is no difference between Ultra Band-Lok and Ketac-with regard to their daily and cumulative fluoride release.

The null hypothesis is rejected as the results of this study show there was a significant difference in both the daily and cumulative amount of fluoride released from each cement.

2. There is no difference between Ultra Band-Lok and Ketac-Cem in relation to their antimicrobial activity against the four strains of Streptococcus mutans tested.

The null hypothesis is rejected as the results of this study show there was a significant difference in antimicrobial activity between the two cements.

3. There is no difference in the extent and position of microleakage when Ultra Band-Lok and Ketac-Cem are used to retain orthodontic bands.

This null hypothesis is accepted for the cement/enamel interface only but there is a suggestion of its rejection for the cement/band interface.

5.2 SUGGESTIONS FOR FURTHER WORK

The results of this study have shown that Ketac-Cem releases more fouride over 40 days and has greater antimicrobial activity over the first 24 hours after mixing, than Ultra Band-Lok. However, correlation of these results to the clinical situation is difficult with regard to demineralisation and the antimicrobial activity in the plaque associated with a banded tooth.

The microleakage assessment carried out as part of this study has shown that microleakage occurs between tooth and cement and there appears to be no difference in the extent and pattern of microleakage between the two cements at the cement/enamel interface. How these results relate to the chances of demineralisation of enamel under a band cemented with one of the two cements, in view of the greater fluoride release and

antimicrobial activity of Ketac-Cem is unknown.

This study has shown that there is a suggestion of a difference between the two cement groups for microleakage at the cement/band interface, although repeating the experiment with a larger sample would be required to determine a positive result. In view of the above observations the following are suggested as further studies to explore these topics further.

1. In vivo study to sequentially sample the plaque associated with bands cemented with the two test cements, to determine the in vivo effect of the fluoride release and antimicrobial activity on Streptococcus mutans numbers in plaque.

2. In vitro study to assess and compare the demineralisation associated beneath bands cemented with Ketac-Cem and Ultra Band-Lok attempting to correlate this with the fluoride release from the two cements.

3. In vivo study to compare demineralization beneath bands cemented with Ketac-Cem and Ultra Band-Lok.

APPENDIX 1

PREPARATION OF TISAB SOLUTION

The following chemicals are mixed in the order shown by stirring;

57ml glacial acetic acid.

58g sodium chloride.

4g CDTA (cyclohexylene dinitrilo tetra acetic acid).

500ml distilled water.

After cooling, 5M sodium hydroxide is added dropwise to adjust the pH value to between 5.0-5.5. Distilled water is then added to make the final solution up to 1 litre volume.

APPENDIX II

PREPARATION OF LOW-LEVEL TISAB SOLUTION

500ml distilled water.

57ml glacial acetic acid.

58g sodium chloride.

All the above are mixed by stirring and the solution allowed to cool. 5M sodium hydroxide is added dropwise until the pH is between 5.0-5.5. The resulting solution is made up to 1litre with distilled water.

APPENDIX III

PREPARATION OF A LOW-LEVEL FLUORIDE CALIBRATION CURVE BY INCREMENTAL ADDITIONS OF LOW LEVEL STANDARD SOLUTION

Step	Added Volume Of Low-level Standard	Concentration Of Fluoride (ppm)
1	0.1ml	0.01
2	0.1ml	0.02
3	0.2ml	0.04
4	0.2ml	0.06
5	0.4ml	0.10
6	2.0ml	0.29
7	2.0ml	0.48

APPENDIX IV

TRYPTIC SOY AGAR CONSTITUENTS:

Peptone

Sodium Chloride

Lecithin

Polysorbate 80

Agar

Water

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