Micro-leakage and Enamel demineralisation:

A comparative study of three different adhesive cements

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Keywords

Micro-leakage

Dye penetration

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Micro-hardness

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Resin modified glass ionomer cement

Composite resin cement

Polyacid-modified composite resin cement

Abstract

Introduction: Micro-leakage and enamel demineralization is still a major challenge in dental practice. It can lead to formation of demineralization lesions around and beneath the adhesive–enamel interface (Mali *et al.*, 2006). Enamel demineralization adjacent to orthodontic brackets is one of the risks associated with orthodontic treatment. The prevention of demineralization during orthodontic treatment is therefore essential for aesthetic reasons and to circumvent the onset of caries.

Aim: To assess micro-leakage and enamel demineralization around orthodontic direct attachments (brackets) using three different orthodontic cements.

Materials and methods: In this *in-vitro* study, intact (non carious) extracted human premolars were used to compare the micro-leakage and enamel demineralization of three different cements (Fuji Ortho LC, Rely X luting 2 and Transbond XT). The dye penetration technique was used to evaluate micro-leakage on extracted human premolars. Micro-hardness testing was performed on 21 teeth to determine enamel demineralization. Sixty teeth were randomly divided into 3 groups of twenty teeth each. Direct attachments were cemented on each tooth using 3 different cements; Fuji Ortho LC (GC Fuji II LC GC Corporation Tokyo, Japan), (group 1), Rely X luting 2 cement (3M ESPE dental product, USA), (group 2), Transbond XT Light Cure (3M Unitek, Monrovia, Calif), (group 3). After the orthodontic direct attachments were fitted, they were exposed to 500 thermo-cycles between 5°C and 55°C, with a dwell time of 15 seconds in a buffered (pH 7) 1% methylene blue dye solution (Grobler *et al*, 2007). The specimens were viewed under a stereomicroscope (Nikon, Japan) at magnification of 40 times. Photographs of each specimen were taken with a Leica camera (Leica DFC 290 micro-systems, Germany) fitted onto a stereomicroscope. The ACDsee photo editing programme was used to transfer the photographs to a computer to measure the dye penetration along the enamel–adhesive and adhesive–bracket interfaces, both on the gingival and occlusal edge at × 40 magnification.

For the demineralization sample, 21 teeth were divided into 3 groups of seven teeth each, where direct attachments were cemented using each of the 3 cements, group 1, Fuji Ortho LC (GC Fuji II LC GC Corporation Tokyo, Japan); group 2, Rely X luting 2 cement (3M ESPE dental product, USA) and group 3, Transbond XT Light Cure (3M Unitek, Monrovia, Calif). A digital hardness tester with Vickers diamond indenter (Zwick RoellIndentec (ZHV; Indentec UK) was used to measure surface micro-hardness of enamel before and after attaching the brackets. Ten indentations were made on the enamel surface of each tooth before bonding the brackets with a 300g load applied for 15 seconds to establish the baseline hardness value. After de-bonding the brackets, the hardness was measured again in the same area as mentioned above to determine the degree of enamel demineralization (softening).

Result: The result showed statistically significantly lower levels of micro-leakage for Transbond XT (P= <0.001). The amount of micro-leakage on the margins was significantly higher in the gingival portion (P <0.05) as compared with the occlusal margin. Enamel micro-hardness tests before bonding using the three different cements showed that the variances are not significantly different (Chi-squared = 3.051, df = 2, p-value = 0.218). However, the micro-hardness tests done after bonding and thermo-cycling was statistically significantly different (Chi-squared = 13.435, df = 2, p-value = 0.001). Clearly, the Transbond XT group had less hardness, implying greater demineralization than the Fuji Ortho LC and Rely X luting 2 groups. Two sample t-tests show that mean value for the Fuji Ortho and Rely X luting 2 were not significantly different from each other (t = -0.636, df = 12, p-value = 0.537). The mean value for Transbond XT differed significantly from both the other two means:

Transbond XT vs Fuji Ortho LC (t = 3.249, df = 6.9, p-value = 0.014). Transbond XT vs Rely X luting 2 (t = 3.493, df = 6.8, p-value = 0.011).

Conclusions: This study showed that Fuji Ortho LC and Rely X luting 2 show more micro-leakage than Transbond XT. However Transbond XT had significant lower micro-leakage, less hardness (greater demineralization) than the Fuji Ortho LC and Rely X luting 2. This may have been due to the fluoride release which significantly reduces demineralization. Therefore the Fuji Ortho LC and Rely X luting 2 may be recommended for prevention of demineralization during orthodontic treatment.

DECLARATION

I hereby declare that “*An In vitro Study of Micro-leakage and Enamel demineralization: A comparative study of three different adhesive cements”* is my own work, that it has not been submitted before for any degree or examination in any university, and that all the sources I have used or quoted have been indicated and acknowledged by complete references.

Student Name: Marrow Elshami

Signed: …………………………

Date: 2016

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DEDICATION

To my mother and my father for their constant support and sacrifice.

To the love of my life Yousef for his constant love and support.

To my supervisor whose guidance, encouragement, help and support made this project possible.

To my loving sisters and my brothers for their constant support.

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1.1 Introduction:

Orthodontic treatment is the rearrangement of teeth in an aesthetic, stable and functional position. This is achieved by the introduction of various types of appliances including direct attachments (brackets) as well as bands and arch wire to establish a good occlusion (Olivia *et al*, 2000; Mason & Kuo, 2010). During orthodontic treatment the posterior teeth are either banded or bonded with the use of bands or direct attachments respectively. In recent times direct attachments are more commonly used as it takes up less clinical time, and it is easier to maintain good oral hygiene thus ensuring increased preservation of gingival health. Orthodontic direct attachments are typically used for treating malocclusions as it gives superior reliability resulting in better resistance to occlusal interference (Fricker, 1997; Tamizharasi & Senthil Kumar, 2010; Moosavi *et al.*, 2013). The orthodontic direct attachments are bonded to the enamel surface of the teeth using orthodontic adhesive cements.

Adhesive materials such as composite resins, conventional glass ionomer cements, resin modified glass ionomer cements and polyacid-modified composites are used to attach the direct attachment to the enamel surface of the tooth (Uysal *et al.*, 2010). Adhesive materials have different polymerization properties due to chemical, light or dual curing (Uysal *et al.*, 2010; Mulder *et al.*, 2013). The orthodontic adhesive cement should have properties such as adequate long working time for placing the bracket in the right position; as well as a short setting time to minimise patient discomfort. Ideally, orthodontic adhesive cements should also be non-irritating to oral mucosa, and have fluoride releasing properties (Patil *et al.*, 2014).

Conventional glass ionomer cements can adhere to enamel and metal, release and uptake fluoride. This cements weakness is its brittleness (Ewoldsen & Demke, 2001). To improve the bond strength and reduce the brittleness of conventional glass ionomer, resin was added (Uysal *et al.*, 2008). Furthermore resin modified glass ionomer cements have fluoride releasing properties and can be used in the presence of moisture (Ewoldsen & Demke, 2001). On the contrary, Polyacid-modified composite resins also have fluoride releasing properties but are moisture sensitive (Patil *et al*, 2014). Although composite resin cements have sufficient bond strength and are easy to handle, they do not have sufficient fluoride release for inhibition of microbial growth and are moisture sensitive (Patil *et al.*, 2014). Additionally, one of the major disadvantages of composite resin cements is polymerisation shrinkage (Ewoldsen & Demke, 2001). Polymerization shrinkage can lead to the formation of micro-gaps between the adhesive cement and the enamel surface of the teeth which contributes to micro-leakage (Cenci, Demarco, & Carvalho, 2005). Micro-leakage also permits the infiltration of micro-organism such as bacteria and oral fluids.

Other factors that may contribute to micro-leakage include thermal expansion and water absorption of adhesive cements (Retief, 1994). In addition, long-term mechanical loading and thermal changes can cause elastic deformation and physical alteration of both tooth substance and restoration, resulting in micro-leakage (Hilton, 2002).

A variety of *in-vitro* methods have been utilised to measure micro-leakage. These include compressed air, neutron activation, fluid filtration, bacteria, electrochemical investigations, scanning electron microscopy and the use of dyes (Kidd, 1976 cited in Vicente *et al*., 2009; Taylor & Lynch, 1992). Perhaps the most commonly used method is that of dye penetration (Hilton, 2002).

Micro-leakage may be a major complication of orthodontic treatment if penetration of microorganisms, fluid and ions occur through the tooth-adhesive interface (Kidd, 1976 cited in Vicente *et al*., 2009). Micro-leakage is definitely an important issue in modern dentistry, particularly when new versions of adhesive materials are introduced into clinical practice. It also leads to severe consequences such as enamel demineralization or white spot lesions at and under the adhesive–enamel interface, enamel discoloration and bond failure (Gorelick *et al.*, 1982 cited by Arhun *et al.*, 2006; Moosavi *et al.*, 2013).

Additionally, the development of demineralization may also be perpetuated by the accumulation of dental plaque and the lack of adequate oral hygiene practices (Artun & Brobakken, 1986 cited by Paschos *et al.*, 2009).

Patient compliance is one of the key contributing factors of enamel demineralisation due to inadequate oral hygiene practices (Behnan *et al*., 2010). Preventative measures such as the addition of fluoride to adhesive cements are said to decrease the rate of demineralization (Gorelick *et al*., 1982; Cohen *et al.*, 2003; Paschos *et al.*, 2009). The addition of fluoride to the adhesive material is important as the fluoride ions absorb onto the surface of the enamel crystals, inhibiting the dissolution rate in acidic conditions, inhibiting bacterial enzymes and promoting remineralisation (Arhun *et al.*, 2006).

There are major differences in micro-leakage studies between operative dentistry and orthodontics. In operative dentistry, the composite resin restoration is thicker and therefore may result in an increase in micro-leakage. In orthodontics, the thinness of the resin results in less micro-leakage (James *et al*., 2003). There are several types of adhesive cements which are routinely used for the application of orthodontic attachments (Bakopoulou *et al*., 2009). The majority of the materials display different degrees of marginal micro-leakage due to changes in material dimension and a lack of good adaptation to the tooth surface (Mali *et al*., 2006). Rely X Luting 2 cement (resin modified glass ionomer) is an adhesive cement that was introduced to the dental market in 1994. Published research on micro-leakage and enamel demineralization of the Rely X Luting 2 cement (resin modified glass ionomer) is sparse. Therefore, the aim of this study was to compare the micro-leakage and demineralization patterns around direct attachments with the use of three different orthodontic direct attachment cements, namely resin modified glass ionomer cement (GIC), other resin-modified glass ionomer cement and composite resin *in vitro*.

Chapter 2

Literature review

Chapter 2

2 Literature review

2.1. Introduction

In direct bonding was proposed in 1965 by Newman as a viable clinical technique. A progress report published in the American Journal of Orthodontics (Rossouw, 2010). Direct bonding is a branch of adhesive dentistry which is designed for bonding metal and ceramic brackets on the enamel surface of the tooth. Various orthodontic adhesive cements such as zinc phosphate, zinc polycarboxylate adhesive cement, glass ionomer cement, resin modified glass ionomer, composite resin and polyacid-modified composite resin (compomer) are used to attach orthodontic attachments to teeth.

Zinc phosphate adhesive cements were first used over a century ago (Boston and Jefferies, 2009; Loher *et al.,* 2009; Fakiha *et al*., 1992), and their development has continued since (Wagh & Arun, 2004). Numerous formulations have been developed and made available to dentists (O'Brien, 2002; Neira *et al.,* 2009; Dickens & Flaim, 2008; Londono *et al*., 2009). As a result, they are used as adhesive cement for fixation of crowns and bridges, inlays, orthodontic bands and attachments (Nicholson *et al.,* 2001).

Polycarboxylate cement was the first chemically cured adhesive cement to chelate to calcium in enamel and dentine, resulting in a chemical bond between the cement and the tooth, due to the carboxyl groups spaced along the polycarboxylic acid chain (Rossouw, 2010). However, its limited use in the orthodontic clinic is due to its relatively high solubility and low fracture resistance (Ewoldsen & Demke, 2001).

Glass ionomer cements (GICs) were first introduced by Wilson and Kent (1972). GICs capitalize on carboxyl chelation to enamel, dentine, and most metals by employing various mixtures of carboxyl-containing acids (polyalkenoic acids) reacting with aluminosilicate glass. Aluminosilicate glass fused in the presence of fluoride fluxes results in an alkaline composition that releases fluoride ions when reacting with acids (Ewoldsen & Demke, 2001).

Glass ionomer cements are now being used quite widely due to its fluoride releasing properties (Bassham, 1999; Uysal *et al.*, 2010). In the late 1980's, glass ionomer cements were proposed for use as an alternative to the more commonly used composite material for orthodontic bonding (Sudjalim *et al.*, 2006).

Resin modified glass ionomer are adhesive cements developed from adding 10% to 20% resin monomers to the GICs which is initially hardened with the use of either light or chemical activators to polymerize the monomers thus resulting in improved physical properties and more stable hydrogels compared with GICs. Resin modified glass ionomer have linked acidic functional groups capable of chelation with the calcium in hydroxyapatite (Van Landuyt *et al*., 2008) which can maintain the ability to bond to the enamel surface (Ferracane *et al*., 2011).

The bisphenol A-glycidyle methacrylate (Bis – GMA) resin was synthesized and introduced by Bowen in 1956. This resin formed the basis of bonding material and the successful production of composite resin which was found to be useful for restorations of the anterior teeth. Additionally, it was ideal for bonding orthodontic attachment to the enamel surface of the teeth (Rossouw, 2010*)*. Composite resin materials are used both in conservative dentistry as a restorative material as well as an orthodontics bonding material. They are made up of two main components: an organic resin matrix and an inorganic mineral filling (Tecco *et al.*, 2005). The inorganic filling content of the material affects polymerization shrinkage, which in turn increases the chance of marginal leakage (James *et al*., 2003). This bonding system is based on the acid etching technique that was introduced in 1955 by Buonocore (Rix *et al.*, 2001).

Buonocore realized that various ions and saliva in the mouth can affect the superficial enamel surface, which makes it different from the underlying enamel. Buonocore (1955) demonstrated that better adhesion was obtained by treating the enamel with surface acid to remove contamination and improve bonding. This is a similar technique used in the paint industry which relies on treatment of metal surfaces with phosphoric acid and phosphoric acid preparations to enhance adhesion effects. This, he believed would render the enamel more receptive to adhesion in a similar manner (Rossouw, 2010). Consequently, resin composite adhesive materials were more viable when coupled with Buonocore's acid-etching technique (Buonocore, 1955) to promote adhesion to enamel (Ferracane *et al.,* 2011).

The acid-etching technique has been used extensively for bonding direct orthodontic attachments with composite resins (Jou *et al.*, 1995; Toledano *et al.*, 2003). This technique facilitates the penetration of resin into the dental tissue. The resin bulk retained in the enamel thus provides the mechanism that mediates the attachment of the orthodontic bracket (Osorio *et al.*, 1998; Toledano *et al.*, 2003).

Polyacid-modified composite resins also known as compomers are adhesive and restorative materials which are a combination of composite and glass ionomer (Ewoldsen & Demke, 2001). Compomers typically consist of mono-, di- or multi-methacrylate monomers, polycarboxylates, as well as some (meth) acrylate monomers bearing pendant carboxylic, phosphoric or related acidic functional groups. Compomers were developed to bring the features of caries inhibition and carboxyl chelation to resins (Ewoldsen & Demke, 2001). However, they release significantly lower levels of fluoride compared with GICs (Grobler *et al*., 1998).

Micro-leakage and enamel demineralization remains a major challenge in dental practice. Micro-leakage may lead to the formation of demineralization lesions around and beneath the adhesive–enamel interface (Mali *et al.*, 2006; Yagci *et al.*, 2010). Enamel demineralization is undesirable as well as unaesthetic but it is not an uncommon complication that may be seen during or after fixed orthodontic appliance treatment (Paschos *et al.*, 2009). There are numerous studies that have described a significant increase in the prevalence and severity of enamel demineralization after orthodontic treatment compared with controls (Chang *et al.*, 1997; Benson *et al.*, 2005). The overall reported prevalence amongst orthodontic patients ranges from 2 to 96 per cent (Arhun *et al.*, 2006).

2.2. Type of orthodontic adhesive cement:

The various types of orthodontic adhesive cement include: zinc phosphate cement, zinc polycarboxylate cement; glass ionomer cement (GIC); resin modified glass ionomer cement (RMGIC); composite resin cement; polyacid-modified composite resin cement (compomer).

2.2.1. Zinc Phosphate cement

Zinc Phosphate was one of the very first permanent cements to emerge onto the dental market, which is a reaction product of zinc oxide and phosphoric acid. The many uses of this cement include permanent cementation of crowns, orthodontic appliances, intraoral splints, inlays, post systems, and fixed partial dentures (Ewoldsen & Demke, 2001).

Zinc phosphate has some advantages such as low solubility in oral fluid, dimensional stability, high compressive strength, moderate tensile strength, and clinically acceptable thin film thickness when applied properly according to the manufacturer's instructions. The major disadvantages are its initial low pH, which has been reported to contribute to pulpal irritation, and its inability to bond chemically to tooth structure (Ewoldsen & Demke, 2001). Therefore, they are not suitable for orthodontic bonding.

2.2.2. Zinc Poly-carboxylate adhesive cement:

Zinc Polycarboxylate cement consists of zinc oxide and a polycarboxylic acid solution. Invented in 1968, it was the first cement to exhibit a chemical bond between the cement and the tooth due to the carboxyl groups spaced along the polycarboxylic acid chain to chelate to calcium in enamel and dentine. The chelation of carboxyl groups to divalent and trivalent cations results in a chemical bond to tooth surfaces and metal surface oxides (Prosser *et al* 1984). The many uses of this cement include permanent cementation of crowns, bridges, inlays, onlays, and orthodontic appliances (Prosser *et al* 1984). However its limited use in the orthodontic clinic is due to its long mixing time, little effect on oral tissue, high solubility, and low fracture resistance (Ewoldsen & Demke, 2001).

2.2.3. Glass ionomer cements (GIC):

GIC have been used in dentistry for a number of years because of its anti-cariogenic effect due to its fluoride releasing properties (Olivia *et al.*, 2000). It is also one of the most effective agents in caries and enamel demineralization or white spot lesion prevention because it encourages remineralisation of porous enamel (Forsten, 1998: Poschos *et al.*, 2009). It has been used for cementing orthodontic appliances because of its ability to chemically adhere to enamel and metal with high compressive and tensile strengths (Poschos *et al.*, 2009).

The main advantage of using GIC as orthodontic adhesive cement is its fluoride releasing property and the fact that it does not require the acid etching procedure. However, is not widely used due to its poor bond strength (Oen *et al.*, 1991: Wilson & Donly, 2001). It has been reported to present a problem in terms of micro-leakage in *in-vitro* studies on the tooth / restoration interface (Bakopoulou *et al.*, 2009). Other disadvantages reported include: initial sensitivity to moisture contamination from the oral cavity, a prolonged setting reaction as well as a late gain in bond strength (Millet & McCabe, 1996). The long setting time results from the poor reactivity of the aluminosilicate glasses in the early cements that produced poor durability and high water absorption and solubility (McLean *et al.*, 1984; Atkinson & Pearson, 1985).

Marcusson *et al.* (1997) compared the ability of GIC to reduce white spot lesion formation with a conventional diacrylate bonding agent. They demonstrated that less white spot lesion formation occurred on teeth bonded with GIC (24%) compared with those bonded with diacrylate (40.5 %).

Benson *et al.* (2004) also showed that the use of glass ionomer cement for orthodontic bracket bonding decreased the prevalence and severity of white spot lesions. Therefore, Glass ionomer cement (GIC) has been considered as an alternative adhesive for bonding orthodontic attachments.

Glass ionomer cements may be classified into three types according to the reactions: conventional (cement setting reaction), dual-cured (chemical setting or light activated reaction), or tri-cured (chemical or light activated polymerization, as well as by a cement setting reaction). The new generation of hybrid glass ionorner materials are dual- or tri-cured and differ considerably in their properties from the conventional glass ionomer materials because they contain resin and glass ionorner components (Wilson & Donly, 2001).

2.2.4. Resin modified GIC:

The more recently developed resin modified GICs differ from conventional GICs, due to the incorporation of resin, water-soluble initiators and activators (Bourke *et al.*, 1992; Wilson & Donly, 2001). These set in part through an acid-based reaction and in part during a polymerization reaction. For this reason it is also referred to as dual-setting resin glass ionomer cement (Bourke *et al.*, 1992; Schmalz, 2009).

2.2.4.1. Properties of resin modified GIC:

The objective in developing this cement was to combine the ease of handling offered by glass ionomer cements with the favourable long working time, better bond strength and greater tolerance of moisture compared with traditional GICs (Mennemeyer *et al.*, 1999; Millett *et al.,* 2009). The development of resin modified glass ionomer cements (RMGICs) also known as hybrid GIC improved the physical characteristics of traditional GICs with the addition of resin. Thus, resulting in better bond strength, fluoride releasing properties, rapid setting reaction by visible light, and enhanced mechanical and physical properties (Creo *et al.*, 1989; Wilson & Donly, 2001).

It is found that although Glass ionomer cements have been improved, clinicians still prefer the use of composite resin cements. It may be because they are more familiar and comfortable with the acid etched technique and the handling characteristics of composite resin cements (Schmalz, 2009).

Resin reinforced or modified glass ionomer cement used in the present study **Fuji Ortho LC** (GC Fuji II LC, GC Corporation Tokyo, Japan) which is a light cured.

It’s available in the form of a powder and liquid within command set system. The complete setting time for Fuji Ortho LC is 4 to 5 min. The sticky consistency of the cement is advantageous as it prevent the bracket from sliding out of position whilst setting.

Fuji Ortho LC powder is composed of fluoroalumino-silicate glass; polyacrylic acid, water, monomer and an activator; 2-hydroxyethyl methacrylate; camphorquinone as a photoinitiator.

The claimed advantages of Fuji Ortho LC include good bonding even in a moist environment; easy application; easy removal of orthodontic appliances avoiding enamel fractures; long term fluoride release; no discoloration, and etching not being a requirement. However, the disadvantages of Fuji Ortho LC are its initial viscosity which may allow some bracket “creep”**;** greater incidence of bracket de-bonding when etching technique not used; less bond strength compared to composite resin (Silverman *et al*., 1995); low abrasion resistance.

The other resin modified glass ionomer cements used in the present study was **Rely X luting 2 cement** (3M ESPE dental product, USA**).** It is composed of two separate pastes dispensed out of the Clicker Dispenser in a 1:1 volume ratio. Rely X Luting 2 Cement is packaged as an easy paste-paste mix, and comes in a double-barrel Clicker dispenser with a mixing time of 20 seconds.

The Rely X Luting 2 cement paste **A** is composed of a radiopaque fluoroaluminosilicate glass (FAS glass); opacifying agent; HEMA; water; reducing agent that allows for the self-cure methacrylate setting, and a dispersion aid.

The RelyX Luting 2 cement Paste **B** is composed of a non-reactive zirconia silica filler; the methacrylatedpolycarboxylic acid; 2-hydroxyethyl methacrylate (HEMA); bisphenol a diglycidyl ether dimethacrylate (Bis-GMA), water and potassium persulfate.

The claimed advantages of Rely X luting 2 cement are good bonding to the tooth surface without the use of a separate tooth conditioner, low solubility; good strength properties; paste formulation for easy mixing; hygienic handling with the double-barrel Clicker dispenser and release fluoride ion.

2.2.5. Composite resin cement:

Resin based composites are widely used as restorative materials as well as for bonding orthodontic brackets in dental practice because of its good bond strength (James *et al*, 2003; Schmalz, 2009).

They became popular and were accepted for bonding orthodontic brackets to teeth (Newman, 1965; Bernstein, 1965; Retief *et al.*, 1970; Schmalz, 2009), following the introduction of the acid etch technique introduced by Buonocore in 1955 (Rix *et al.*, 2001; Toledano *et al.*, 2003).

The acid etching technique has been used extensively for bonding direct orthodontic attachments with composite resins (Jou *et al.*, 1995; Toledano *et al.*, 2003). This technique facilitates the penetration of resin into the dental tissue. The resin bulk retained in the enamel provides the mechanism that mediates the attachment of the orthodontic bracket. However, the acid-etching technique produces some undesirable effects like the risk of enamel demineralization, and enamel loss when the composite residue is removed using burs or disks (Osorio *et al.*, 1998; Toledano *et al.*, 2003).

2.2.5.1. Composition of Composite resin cement:

The composite resin cement consists of an organic resin-based matrix such as a bisphenol A-glycidyl methacrylate (BIS-GMA) resin and inorganic filler such as quartz and silica. The filler gives the composite wear resistance and translucency (Schmalz, 2009).

It has great clinical approval both for bonding of orthodontic attachments and for restorative purposes. However, it has many disadvantages including loss of enamel during acid etching, enamel demineralization around the attachment as well as polymerization shrinkage (Pradeep *et al.,* 2013).

Additionally, Composite placement is technique sensitive, as moisture contamination, may compromise adhesion to tooth structure (Yazici *et al.*, 1985; Rix *et al.*, 2001; Patil *et al.*, 2014). Increase in durability and the prevention of micro-leakage is achieved only if the adhesive is well bonded at the tooth material interface (Yazici *et al.*, 2002). Numerous adhesive bonding agents were developed after the introduction of the acid-etch technique including chemical and light activated products. Chemical-curing (CC) constitutes polymerization of benzoylperoxide (BP) (the initiator) and an aromatic tertiary amine activator which initiates setting of the composite resin (Hanks *et al*, 1988). However, the disadvantage of the chemical-cured adhesive system is the limited setting time (Joseph & Rossouw, 1990; Toledano *et al*, 2003). Later, composite bonding systems incorporated fluoride in an attempt to reduce the risk of developing white spot lesions or enamel demineralization. However, laboratory studies concluded that initial high fluoride release was short-lived and was unable to prevent enamel demineralization during the course of orthodontic treatment (Bishara *et al.*, 2005).

Tavas & Watts (1979) were the first to report on an in vitro study on use of light-cured (LC) materials for bonding orthodontic attachments. They suggested that the tooth structure transmits visible light and thus the material is cured under metal-based brackets by direct illumination from different sides and by transillumination. Rapid polymerization occurs when visible light is applied, producing a ‘‘command set’’. A great advantage of this system is that it affords nearly unlimited working time, thus allowing more accurate orthodontic bracket placement (Trimpeneers *et al.*, 1996; Toledano *et al.*, 2003).

The polymerization shrinkage percentage of most available resin-based composite systems ranges from 1.4% and 5.67% (Mulder *et al*., 2013). The variation in shrinkage from composite to composite depends on the percentage of filler, the diluents, and the percentage of monomer conversion in the specific composite resin (Burgess *et al*., 1999; Mulder *et al.,* 2013). It increases as the filler content decreases (Miyazaki *et al.*, 1991; Arhun *et al.*, 2006).

Light cure adhesives are the most popular due to its high primary bond strength, better physical properties, user friendly application, long working time and easier removal of adhesive excess. Disadvantages of light cure adhesives may be that they are time-consuming, light transmission may be hindered, and polymerization shrinkage may occur (James *et al.*, 2003).

Composite resin used in the present study was **Transbond XT Light Cure Orthodontic Adhesive cement (3M Unitek, Monrovia, Calif)**

Transbond XT bonding system comprises of the Light cure adhesive primer and an Adhesive Paste. The adhesive uses light cure adhesive technology providing additional working time to ensure accurate bracket placement.

Transbond XT adhesive cement is composed of silane-treated quartz**;** bisphenol a diglycidyl ether dimethacrylate**;** bisphenol a bis (2-hydroxyethyl ether) dimethacrylate**;** dichlorodimethylsilane reaction product with silica.

The claimed advantages of Transbond XT adhesive cement is its viscosity, which may prevent bracket skating and reducing adhesive waste; its ability to bond with ceramic and metal brackets; good handling properties and it’s easy to removal.

2.2.6. Polyacid-modified composite resins

Polyacid-modified composite resins, also known as Compomers, have good features of caries inhibition and carboxyl chelation to resins. Compomers are single-component systems consisting of aluminosilicate glass in the presence of carboxyl modified resin monomers and light-activated conventional resin monomers. Although the alkaline glass and acidic carboxyl components are packaged in the same container, allegedly no acid-base setting reaction occurs because water is absent from the composition (Ewoldsen & Demke, 2001).

Compomer adhesives have more properties that are similar to resin adhesives than they have to RMGIC; they bond primarily through physical interaction with dry surfaces. They release less fluoride than that of GICs but higher than that of resin adhesives (Ewoldsen & Demke, 2001).

2.2.7. Comparison of adhesives for orthodontic use:

This information is proposed to explain the ideal chemical and physical properties of various orthodontic bonding materials. Clinicians need to be well-informed about the orthodontic bonding adhesives such as zinc phosphate cement, zinc polycarboxylate cement; glass ionomer cement GIC; resin modified glass ionomer RMGIC; composite resin; polyacid-modified composite resin (compomer), so that they can select and use these bonding materials appropriately (Ewoldsen & Demke, 2001).

Polycarboxylate cement can chemically bond to tooth and metal surfaces however, its limited use in orthodontics is due to its long mixing time, high solubility, low fracture resistance (Prosser *et al*., 1984; Ewoldsen & Demke, 2001).

Glass ionomer cements (GICs) have desirable properties including fluoride release around orthodontic attachments and also the ability to chemically bond to tooth structure (Arthun & Bergland, 1984). However, they are not deal for bonding orthodontic brackets due to their brittle nature, low fracture resistance and low tensile strength (Prosser *et al.,* 1984; Ewoldsen & Demke, 2001). GIC's can inhibit demineralization and its improved band retention are the chief reasons it remains useful to orthodontist for cementing bands in high caries-risk patients (Fricker, 1989; Ewoldsen & Demke, 2001). Despite the low bracket-retention rates of GICs, their chemical adhesion and moisture tolerance eliminate the need for acid etching and drying (Ewoldsen & Demke, 2001). In order to address the short comings of GICs, Resin modified glass ionomer cements (RMGIC) were developed with improved properties that make them very effective for orthodontic bonding. These properties include fluoride release and good bond strength (McCourt *et al.,* 1991).

The most frequently used orthodontic bonding adhesive is found to be composite resins, (Schmalz, 2009) due to its bond strength and increased durability (Yazici *et al.*, 2002). However, the procedure requires a dry field, it has no fluoride releasing properties and undergo polymerization shrinkage (James *et al.*, 2003). Other undesirable effects include the risk of enamel demineralization during acid etching; the risk of enamel fractures during de-bonding and enamel loss when the composite residue is removed using burs or disks (Osorio *et al.*, 1998; Toledano *et al.*, 2003).

Polyacid-modified composite resin used as adhesive cement can prevent caries formation due to fluoride release from the aluminosilicate glass filler at low pH. Fluoride release from Polyacid-modified composite resin is lower than that from GICs but higher than that from resins. However, it does require acid etching during bonding procedure and a dry field (Tate et al., 2000).

2.3. Ideal properties of orthodontic adhesive:

Orthodontic adhesive should be capable of enabling brackets to stay bonded to the enamel for the whole duration of treatment and to permit easy removal of brackets when it is needed, without causing damage to the enamel surface (Patil *et al*., 2014).The adhesive should be non-irritating to the oral mucosa, allow adequately long working time for positioning brackets while setting quickly for patient's comfort. In addition, it should provide a simple way of application and a convenient way of curing (Patil *et al*., 2014), it should have ease of use, good bond strength, moisture tolerance, as well as fluoride releasing properties (Rix *et al.*, 2001; Goje *et al.*, 2012). Generally orthodontic treatment extends over a prolonged period of time. This increases the risk of demineralization and caries. Therefore, the use of fluoride containing adhesive to bond the brackets can be extremely beneficial.

2.4. Challenges of dental adhesive

The major challenges of using orthodontic adhesive for bonding include:

- Challenges relating to the adhesive itself are polymerization shrinkage, thermal expansion and mechanical stress,
- Challenges relating to bonding are bond strength failure, micro-leakage and white spot lesion formation (demineralization).

2.4.1. Polymerization shrinkage:

The decrease in distance between groups of atoms/molecules with resulting volume change during polymerization is referred to as shrinkage (Mulder *et al.*, 2013). Polymerization shrinkage is defined as the ratio of the change in volume to the original volume respectively, producing stress and forming gaps (Ensaff *et al.*, 2001). This phenomenon is observed in both orthodontic bonding as well as in restorative dentistry.

Polymerization shrinkage is one of the most critical properties to consider in aesthetic resin composites as it is one of the key factors that may contribute to micro-leakage. This results from the variability of the adhesive cement composition, percentage of filler, the diluents, or the percentage of the monomer conversion in the specific composite resin as well as the curing method (Oberholzer *et al.,* 2005; Mulder *et al.*, 2013). During polymerization, shrinkage may occur because the monomer is closer to one another than they were in the original monomer state (Chen *et al.*, 2001). Polymerization shrinkage as low as 2% may produce enough tension to destroy the marginal integrity between the material and the tooth structure resulting in micro-leakage, post-operative sensitivity and/or bond failure (Mulder *et al.*, 2013).

The contraction stress build-up that occurs during polymerization, leads to an unfavourable interface between composite resins and enamel, potentially causing de-bonding (Chen *et al.*, 2001). The amount of curing contraction stress build-up which is generated by light-curing bonded composite resins is also an important factor contributing to the longevity of the composite material.

Generally, the shrinkage can resolve in the early plastic state (before the polymerization gel point) by flow, or minimizing contraction stresses by allowing the composite volume to change shape. The polymerization process is then accompanied by a rapid increase in the elastic modulus in the polymerization gel formation. Contraction stress build-up which occurs following shrinkage is obstructed as the material is rigid enough to resist sufficient plastic flow to move back to the original volume (Chen *et al.*, 2001).

2.4.2. Thermal expansion and mechanical stress

Thermal stresses may possibly be problematic in two ways. Firstly, differential thermal changes can induce mechanical stresses and lead to crack propagation through the bonded interface. Secondly, gap volume changes causing changes in gap dimensions. Changes in gap dimensions pump pathogenic oral fluids in and out of the gaps which may result in pulpal complications (Gale & Darvell, 1999).

Restorative materials in the oral cavity environment is constantly exposed to temperature changes because of intake of food and fluids with varying temperatures. Such changes, if significant, can lead to unfavourable effects on the margins of the restorations such as compromising the seal at the tooth-restoration interface (Sidhu *et al.,* 2004).

Restorative materials tend to expand and contract differently when compared with tooth structure. Such expansion and contraction develops stresses at the tooth restorative interface and if the bond or restoration is not able to tolerate the changes this may lead to de-bonding and gap formation (Agosta & Estafan, 2003; Sideridou *et al.,* 2004; Sidhu *et al*., 2004).

2.4.3. Bond strength failure

There are several factors which may play a role in affecting the bond strength of orthodontic attachments. These include inadequate preparation of the tooth surfaces prior to bonding, and moisture or saliva contamination (Rix *et al.*, 2001). Furthermore, inadequate curing time for the light cured materials may also contribute to bond strength failure.

Rossouw *et al*. (1996) demonstrated the effect on bond strength when bracket bases were exposed to contaminants used during orthodontic treatment, which included dental wax, dust powder from gloves, sandblasting powder not properly removed, skin oil, and saliva. They suggested that great care should be taken during preparation for bonding to ensure a successful outcome of the orthodontic attachment bonding process (Rossouw*,* 2010*)*.

Good bond strength can prevent the penetration of fluids and bacteria under the orthodontic attachment and decrease micro-leakage. However, this relationship is not confirmed by evidence based research in orthodontics (Arhun *et al.,* 2006). There are controversial reports regarding the relationship of micro-leakage with bond strength (Arhun *et al.,* 2006; Abdelnaby *et al.*, 2010; Moosavi *et al.*, 2013). Although James *et al.* in (2003) showed that there is no correlation between the micro-leakage and bond strength.

2.4.4. Micro-leakage

Micro-leakage is defined as the penetration of bacteria, fluids, molecules, or ions between the tooth structure and restorations (Kidd, 1976).

Micro-leakage from an orthodontic point of view is considered to be a contributing factor in the formation of white spot lesions at the enamel and adhesive interface around the orthodontic attachment (Arhun *et al.*, 2006; Sabzevari *et al.*, 2013).

Although micro-leakage around orthodontic brackets and bands is reported to be minimally detected (Gillgrass *et al.*, 1999; James *et al.*, 2003; Arhun *et al.*, 2006; Arikan *et al.*, 2006) it is worthy to note the differences in the micro-leakage patterns between various materials.

Numerous studies have been conducted comparing micro-leakage patterns using various available orthodontic adhesive cements. Ramoglu *et al.* (2009) investigated micro-leakage patterns comparing light cured resin modified glass ionomer cement (RMGIC) and composite resin under orthodontic brackets. They indicated that RMGIC had more micro-leakage than conventional composites.

In 2010, Uysal *et al.* compared micro-leakage patterns of conventional glass ionomer cement (GIC), RMGIC, and polyacid-modified composite resin (PAMCR) for band cementation and found that conventional GIC had higher micro-leakage than RMGIC and PAMCR. From the above studies it appears as though the GICs tend to demonstrate more micro-leakage when compared with composite based adhesive.

2.4.4.1. Contributing factors:

Micro-leakage results predominantly in the infiltration of bacteria, fluids, ions between the cement and the tooth structure (Kidd, 1976). Many factors contribute to the development of micro-leakage during orthodontic treatment. These include dimensional changes of materials as result polymerisation shrinkage, thermal contraction, water absorption, mechanical stress and dimensional changes in tooth structure (Staninec *et al*., 1986; Davari *et al.*, 2012).

The polymerisation shrinkage of a composite resin can create contraction forces which can disrupt the bond to the cavity walls, leading to marginal failure and subsequent micro-leakage (Davidson *et al*., 1984). Another reported contributing factor is temperature variations, which is a stressor to the adhesive. Adhesive materials are constantly exposed to changes in temperature in the oral cavity; therefore it can be contribute to the degree of micro-leakage (Bishara *et al.*, 2003; Sabzevari *et al.*, 2013). Thonemann *et al* (1997) have shown that over time, water sorption can cause gap reduction by hygroscopic expansion of resin-based composites. However, the problem of micro-leakage cannot be solved by this mechanism.

Furthermore, Arhun *et al.* (2006) assessed the micro-leakage of a tooth–adhesive–bracket complex with conventional and antibacterial adhesive bonded to metallic and ceramic brackets to the teeth. They found that the metallic brackets had more leakage between the adhesive-bracket interfaces when compared with the ceramic brackets.

From the orthodontic point of view, increase in leakage consequently leads to lower clinical shear bond strength and an increased susceptibility to white spot lesion formation (Arhun *et al.,* 2006). Whereas in restorative dentistry; micro-leakage increases the risk of developing recurrent caries and post-operative sensitivity (James *et al.*, 2003).

2.4.4.2. Prevention of micro-leakage:

A review of the literature by Arhun *et al.* (2006) showed that in orthodontic treatment a thin layer of composite resin can absorb some shrinkage at the edges of the bracket. This shrinkage can pull the bracket closer to the enamel by the free floating bracket which results in less micro-leakage.

Flowable composite resins have a substantially lower modulus of elasticity which may increase elastic deformation and absorb polymerization shrinkage stresses (Fabianelli *et al*., 2007). In addition, flowable composite resins exhibit similar coefficient of thermal expansion to that of tooth structure, which is able to reduce micro-leakage and reduce stress by 18-50 %. However, they cannot prevent micro-leakage completely (Fabianelli *et al*., 2007). Several other new flowable materials have also been introduced into the market for orthodontic use, such as compomers (Bishara *et al.,* 2001; Tecco *et al.,* 2005; Vicente *et al.,* 2006), and giomers (Vicente *et al.,* 2006).

Conventional Glass-ionomer cements exhibit beneficial properties including anti-microbial activity, fluoride releasing properties, adhesion to tooth surfaces when used to bond orthodontic attachments, a reduced failure rate and prevents premature demineralisation, minimising secondary caries formation (Herrera *et al*., 2000).

2.4.4.3. Adverse effects of micro-leakage:

Micro-leakage between the adhesive material and enamel surface may result from polymerization shrinkage of the adhesive material, which can result in severe consequences such as bond failure, increased risk of enamel demineralization and formation of white spot lesions around the bracket (James *et al.*, 2003; Yagci *et al.*, 2010).

Enamel demineralization or the development of white spot lesions can be caused by retained bacterial plaque on the enamel for a prolonged period (Gorelick *et al.*,1982 cited in Paschos *et al.*, 2009) which may permit the infiltration of bacteria and fluids from the oral cavity (Georges *et al*., 2002; Yagci *et al.*, 2010).

2.4.4.4. Measuring Micro-leakage:

Micro-leakage may be assessed using various *in vivo* and *in vitro* methods with or without thermal cycling (Uysal *et al*, 2010). Thermal cycling is often used in manufacturing processes which provides results that correlate laboratory findings more accurately (Mathew *et al.*, 2001; Majeed, 2005). The adhesive materials are exposed to thermo-cycling tests for simulation of oral thermal cycles (Gale & Darvell, 1999; Vicente *et al.*, 2009). In 2003, Wahab *et al.* showed that thermal changes in the mouth may lead to unequal volume changes and subsequently de-bonding at the bonding area because the linear thermal expansion of tooth structure and adhesives are different, hence thermo-cycling tests may decrease bond strength and increase micro-leakage at interfaces (Wahab *et al.*, 2003).

The methods used to assess the micro-leakage include use of staining; scanning electron microscopy; bacterial activity; air pressure; chemical agents; neutron activation analysis; radioisotopes; ionization; autoradiography and reversible radioactive adsorption (Tjan & Tan, 1991; Yavuz & Aydin, 2005). The most commonly used method of assessing micro-leakage in in-vitro detection is the dye penetration technique (Al-Ehaideb & Mohammed, 2001; Uysal *et al.,* 2010).

2.4.4.5. Techniques:

1. Dye penetration:

The Dye penetration technique involves exposure of the study sample to a dye solution, for a determined period (approximately for 24 hours). This is followed by washing and sectioning the specimen and its examination under a light microscope to determine the extent of leakage around the tooth and material interface. This method shows the leakage in contrasting colours to both tooth and material without the need for further chemical reaction or exposure to potentially hazardous radiation. The dye penetration technique is highly sensitive and is the most preferred method (Alani & Toh, 1997). The result of the assessment obtained from the dye penetration technique also goes through a rigorous standardization process due to the possibility of variation in the dentine permeability in the different specimens (Shortall, 1982; Gale; Darvell, 1999), which makes this technique reliable (Shortall, 1982: Taylor & Lynch, 1992). In addition, the specimen is destroyed for evaluation (Youngson, 1992; Wibowo & Stockton, 2001). There are variations in choice of dye used, either as solutions or particle suspensions of different particle size. The concentrations of dye used also ranges between 0.5%-10%, while the time of immersion of specimens in the dye varied between 4 hours to 72 hours or more. It was found that different concentrations of dyes can vary in penetration time between 5 minutes to over 1 hour (Christen & Mitchell, 1966). The organic dyes used in the dye penetration technique are basic fuchsin (Fuks *et al.*, 1992), methylene blue (Prati *et al*, 1991), eosin (Youngson *et al*., 1990), aniline blue (Kakar & Subramanian, 1963), crystal violet (Chan & Swift, 1991) and erythrosine B (Phair & Fuller, 1985). The concentration of dye solutions currently in use for micro-leakage assessment ranges from 0.5% to 2%. Although it does have some disadvantages, it is still accurate, easy to use, of low cost and a comparable method of evaluating micro-leakage (Uysal *et al.*, 2010).

2. Scanning Electron Microscopy

The scanning electron microscopy technique provides direct visual observation of the adaptation of restorative materials to cavity margins and to detect crack defects created during the finishing of composites (Ferracane *et al.,* 1992) due to its high magnification and depth of focus (Boyde & Knight, 1969). Many investigators have used scanning electron microscopy to measure gap formation that occurred between the restorations and walls and floor of the preparation (Davila *et al.,* 1986, 1988; Van Dijken & Horsted, 1989). However, it was criticized for its potential for introducing errors and artifacts related to drying, cracking, distortion, and sectioning (Kidd, 1976). It is also difficult to quantify SEM results and the technique is limited to demonstrating marginal defects (Barnes, 1977).

3. Bacterial activity

The bacterial activity method is used to evaluate the micro-leakage through the use of bacterial cultures. It is considered to be more reliable and clinically relevant than the other methods (Siqueira *et al.,* 2001). The bacterial activity method is considered as one of the best methods for evaluating leakage. This method was established in the field of restorative dentistry in 1965 by Mortensen *et al.* However, this method is criticized, and limited to use due to its inability to simulate conditions in the oral cavity, such as temperature variations, dietary influences, and salivary flow (Alani, 1990).

4. Air Pressure

Another method for detecting marginal micro-leakage patterns involves the use of air pressure. This method was done by constructing class 2 amalgam restorations in a steel dye, distributed air under pressure to the floor of the cavity, and examined the restoration under water (Harper, 1912). Microscopic examination of the release of air bubbles from the margin of the submerged restoration provided a subjective view of the marginal seal. Moller *et al.* (1983) have demonstrated that the air pressure method is a valuable technique for comparing the sealing properties of different amalgams as well as cements. The advantages of the method are that the results can be quantified, and the examination of the specimen does not necessitate its destruction. They were, therefore, able to study leakage over a period of time for the same restoration. The main disadvantage of this method was that it could only detect micro-leakage pathways that were complete from the floor to the margin of the cavity (Taylor & Lynch, 1992).

5. Chemical agent

The use of the chemical agent method to evaluate micro-leakage has benefits because it provides more objective measurements and quantitative data could be collected for which parametric statistical analysis is appropriate (Crim, 1987). However it has similar problems to the dye penetration method in the interpretation of results (Alani & Toh, 1997). A lead glass chemical agent is used to assess leakage around acrylic restorations by incorporating the lead glass in the acrylic, so that when immersed in a solution of barium sulphide, the areas of marginal discoloration would indicate leakage (Kornfield, 1953).

The silver nitrate method of measuring micro-leakage described by Hammesfahr *et al*. (1987) is an acceptable technique, however, it is a severe test because the silver ion is extremely small (0.059 nm) when compared to the size of a typical bacterium (0.5-1.0 μm) and thus is more penetrative.

6. Neutron Activation Analysis

The neutron activation analysis method was used to assess micro-leakage *in vitro* (Going *et al*., 1968). It was done by the immersion of restored teeth in an aqueous solution of a non-radioactive manganese salt. All of the salt adhering to the outside of the tooth is then removed and the whole tooth placed in the core of a nuclear reactor. This resulted in the non-radioactive Mn55 being activated to Mn56 and the x-ray emission of Mn56 formed during irradiation was then measured. The number of radioactive counts is proportional to the uptake of Mn per tooth (Alani & Toh, 1997). Douglas *et al.* (1980) used this method to prove the ability of a hydrophobic composite material to reduce marginal leakage compared to a conventional composite control. This method had an advantage because the results could be quantified. However, the limitations included very high costs, complexity of the method and inability to identify the point at which the restoration leaked (Going, 1972). Serial sections were made to define the path and depth of tracer penetration and this sectioning may create a radiation hazard. Meyer *et al.* (1974) has showed that the presence of manganese, either in the restorative material or in the tooth, caused variability of the results.

7. Radioisotope

Radioisotope is a common *in vitro* method for detecting micro-leakage which involves the use of extracted restored teeth. The advantage of using radioisotopes is detection of minute amounts of leakage, as the smaller isotope molecules measure only 40 nm compared to the smaller dye particles (120 nm) (Going, 1964) and deep penetration into defects (Alani, 1990). However, the main disadvantage of tracer studies are; results evaluated subjectively, complex, needs precautions in handling as elaborate precautions have to be devised to satisfy safety requirements at all stages of the procedure. Additionally, it is an expensive, destructive and sensitive technique with the occasional difficulty in interpretation arising from the possibility of isotope penetration by a route other than the tooth/filling interface (i.e. via cracks in the enamel of extracted teeth used during the study) (Alani, 1990).

The tracers used in radioisotope are Ca45 (Armstrong & Simon, 1951; Hembree & Andrews, 1978; Crim *et al.,* 1985; Puckett *et al.,* 1995), C14 (Cantwell *et al.,* 1959; Powis *et al.,* 1988), P31 (Going *et al.,* 1960; Baumgartner *et al.,* 1963; Galan *et al.,* 1976), S35 (Barber *et al.,* 1964), and Na22 (Briannstrom & Soremark, 1962). In general Ca45 in the form of calcium chloride at a concentration of 0.1 m Ci/ml, is the most popular isotope used, due to possession of low-energy beta emission and it does not readily penetrate enamel (Going, 1972).

2.5 White spot lesion (Enamel demineralization):

Enamel demineralization or white spot lesion (WSL) is defined as the first sign of a caries like lesion on enamel that can be detected with the naked eye (Summitt *et al.*, 2006). The WSL has also been defined as “subsurface enamel porosity from carious demineralization” that presents itself as “a milky white opacity when located on smooth surfaces” (Summitt *et al.,* 2006; Bishara *et al.,* 2008).

Initial enamel demineralization usually manifests itself clinically as a “white spot lesion” (WSL) (Bishara *et al.,* 2008). Enamel demineralization around the orthodontic attachment is an important and prevalent iatrogenic effect of orthodontic treatment. Orthodontic attachments on tooth surfaces lead to an increased susceptibility to plaque retention which also leads to a decrease in pH in the oral environments. This reduce the remineralization process thus giving rise to further enamel demineralization (Øgaard *et al*., 2001; Bishara *et al.,* 2008).

The white spot lesion (WSL) has a low progress to cavitation, occurring in only 2% of white spot lesions (WSL). The high incidence of white spot lesions (WSL) is an important factor in orthodontic treatment especially when the final outcome is to achieve improved aesthetics (Banks *et al*., 2000). Cavitation normally occurs, after long periods of mineral loss and short periods of remineralisation. An endpoint is reached where the surface cannot be reconstituted by re-precipitation from the subsurface (Chang *et al.*, 1997; Higham, 2014). At this stage, the lesion is past the point of spontaneous repair, and thus restorative intervention becomes necessary.

If there is no cavitation, the enamel surface can remain intact and reversal of the demineralization can occur. This process may occur spontaneously, due to the combined action of salivary minerals and fluoride intake from a dentifrice or it may be brought about by therapeutic intervention (Chang *et al.*, 1997; Namboori *et al.*, 2012). Although the area around a bracket is critical to the development of demineralization, the area beneath the bracket also has the potential to develop demineralization (Arhun *et al.*, 2006).

In-vitro studies performed by numerous researchers (Vorhies *et al.*, 1998; Millett *et al.*, 1999; Chung *et al.*, 1999) showed that glass ionomer cements provided a sustained fluoride release for a year or two thus potentially reducing demineralization (Vorhies *et al.*, 1998; Millett *et al.*, 1999; Chung *et al.*, 1999).

Demineralization of the labial surfaces of the teeth during orthodontic treatment is a clinical problem and will affect the aesthetic appearance of the patient and compromise the benefit of orthodontic treatment (Zimmer & Rollwinkel, 2004). So therefore, assessment of white spot lesion susceptibility before orthodontic treatment seems good and sensible. To identify the patients at risk of demineralization, various factors need to be examined (Newbrun, 1989: Lundstrom & Krasse, 1987: Fejerskov & Manji, 1990; Higham, 2014). These factors may include: assessment of salivary flow rate; history of past enamel caries and caries incidence (number of new lesions) over the past year; residence in fluoridated or non- fluoridated communities; dietary patterns; plaque scores; microbial monitoring using salivary *mutant's streptococci* and *lactobacillus* counts (Higham, 2014).

In an attempt to minimize or avoid the enamel demineralization, constant exposure of topical fluoride has been reported to be used to prevent enamel demineralization. The presence of topical fluoride in the adhesive cement is said to acts as a fluoride reservoir that can increase the level of fluoride in saliva, plaque and teeth (Boyles, 2007; Paschos *et al.*, 2009). The release of fluoride from the adhesive cement whether short- term or long-term is dependent on their matrices, setting reaction and fluoride content (Boyles, 2007).

Thus it is important to note that the characteristics and component of adhesive cements can play critical roles in micro-leakage and enamel demineralization.

2.5.1. Prevalence of white spot lesion:

Enamel demineralization or white spot lesions (WSL) have been reported to occur in 2% to 96% of orthodontic patients (Arhun *et al.*, 2006).

It has been reported to occur as early as within 4 weeks with inadequate oral hygiene (Hadler-Olsen *et al.*, 2012; Martignon *et al.*, 2010; and Moosavi *et al.*, 2013).

Gorelick and co-workers (1982) compared the prevalence of enamel demineralisation in patients undergoing orthodontic treatment with a group of controls. They found that twice as many patients in the study group (50%) had demineralization when compared with control group (25%).

This was further supported by Ogaard's (1989) study showing that the incidence of white spot lesions increased in orthodontic patients even five years after treatment when compared with a control group of patients who had not had orthodontic treatment. Gorelick *et al.* (1982) and Ogaard's (1989) findings are further supported by Vorhies *et al.* (1998) where they reported the incidence of white spot lesions on teeth that were bonded with orthodontic appliance was higher than a control group of untreated patients. Their study showed that 49.6 percent of the study sample developed areas of decalcification.

When looking at the susceptibility of specific teeth to white spot formation on bonded teeth it was found to be in the following order: maxillary lateral, mandibular canine, mandibular first premolar, and mandibular first molar, mandibular second premolar, maxillary canine and maxillary first premolar (Vorhies *et al.*, 1998).

2.5.2. **Formation of White spot lesion (demineralization):**

Plaque retentive areas increase following the introduction of orthodontic appliances, thus leading to a rapid shift in the composition of bacteria flora (acidogenic bacteria such as *Streptococcus Mutans*) (Bishara *et al.,* 2008).

Chatterjee *et al*. and Gwinnett *et al.* both in (1979) have demonstrated that patients undergoing orthodontic treatment have increased plaque volume. They have also been shown to have a reduced pH when compared with non-orthodontic patients.

As a result of the lower plaque pH, remineralization is hindered and demineralization is visualized as a WSL (this is the first clinical evidence of demineralization). Clinically, WSL becomes visible within a span of 4 weeks 8 (Bishara *et al.,* 2008). If the highly cariogenic environment around or under the orthodontic appliances is left untreated, it will produce tooth decay, tooth discoloration and compromise the aesthetics (Bishara *et al.,* 2008).

2.5.3. Features of enamel demineralization:

The subsurface mineral loss with an increase in porosity and consequential changes in the optical properties of the enamel leads to an opaque white appearance of the enamel lesion; therefore the surface appears chalky (Chang *et al.*, 1997; Aghoutan *et al.*, 2015).

The white spot lesions arise from a series of repeated episodes of mineral loss from the enamel surface into the plaque and saliva together. This is an interrupted process, as the dynamics of repair and destruction changes according to the oral environment (Chang *et al.*, 1997; Higham, 2014). Thus any fluctuations in pH at the interface between the enamel surface and the plaque will directly influence the diffusion of calcium and phosphate ions out of the enamel, as does the concentration of fluoride at the interface (Chang *et al.*, 1997; Higham, 2014).

Boyd (2001) showed that mild decalcification caused by orthodontic treatment is characterised by white or white-yellow stains or colour change with possible surface roughness. Moderate decalcification is usually seen as larger areas of yellow-brown stain with definite surface roughness whereas severe decalcification is characterised by large areas of darker, yellow-brown stains of clinical colour changes with enamel loss.

2.5.4. Contributing factors:

The contributing factor or the aetiology of enamel demineralization during orthodontic treatment includes:

- Oral hygiene and practices
- Diet
- Microbial factors
- Salivary factors

1. **Oral hygiene and practices**

The accumulation of Plaque causes production of acid and in the presence of fermentable substrate prevents remineralisation by calcium and phosphate ions from the saliva via physical barrier of plaque which limits the diffusion of acid away from the enamel surface. Plaque accumulation and caries promoting bacteria tends to increase during orthodontic treatment; these changes may counteract the tendency for re-mineralization to occur (Chang *et al.*, 1997).

The insertion of orthodontic appliances makes cleaning the teeth more challenging as there are more plaque retentive areas on the tooth surfaces both around the attachments and between it and the gingival margin. Also, fixed appliances may compromise oral clearance by restricting the movement of the tongue to remove food particles from the mouth contributing to further formation and accumulation of plaque (Chadwick *et al.*, 2005). The irregularity of the bracket and band surfaces can decrease the access and buffering by saliva as well as restrict the movement of oral musculature (physical force) to clean, thus leading to lowering of the plaque pH in the presence of carbohydrates. It also creates stagnation areas in between the teeth reducing access by saliva, encouraging a lowering of plaque pH in the presence of carbohydrates and promotes plaque accumulation. Therefore, the decrease in salivary flow will promote demineralization (Chang *et al.*, 1997).

Additionally plaque accumulation tends to occur more readily on the adhesive materials than on the enamel surfaces (Smales, 1981; Wilson & Donly, 2001). As it is more difficult to remove plaque around orthodontic brackets it may lead to more adhesion of bacteria to the adhesive material and thus may contribute to demineralization around the orthodontic appliance (Gwinnett & Ceen, 1979; Wilson & Donly, 2001).

2. Diet

Diet can play a significant role in the demineralization process depending on the frequency of carbohydrate consumption (Aghoutan *et al.*, 2015).

The ingestion of fermentable substrates leads to a decrease in the pH of plaque fluid resulting in overlapping episodes of acid challenge. The frequency of carbohydrate consumption results in continuous pH fluctuations, thus causing the enamel surface to be rapidly demineralized and consequently reducing repair (Chang *et al.*, 1997; Aghoutan *et al*., 2015).

3. Microbial factors

The presence of orthodontic attachment serves as a new retentive site in the mouth. Lundstrom & Krasse (1987) found an increased proliferation of strains of *Streptococcus Mutans* and *lactobacilli* in patients who had orthodontic treatment. *Streptococcus Mutans* synthesize extracellular glucans from dietary sucrose, which may increase the plaque mass and the cariogenicity of plaque. In additionally, there is an increase in the colonization of *Streptococcus Mutants* changing the diffusion properties of the plaque matrix. These changes in the oral cavity can lead to the increased level of colonization of *Streptococcus Mutans* as the retentive areas of plaque surfaces increases the risk of caries (Klock & Krasse, 1979; Arhun *et al.*, 2006). *Lactobacilli*, like *Streptococcus Mutans* are acidouric and acidogenic and when present in large numbers creates the ideal conditions for producing dental caries (VanHoute, 1980: Chang *et al.*, 1997; Namboori *et al*., 2012). However, *Lactobacilli* do not a play role in the initiation of caries (Chang *et al.*, 1997). An *in vivo* study by Hallgren *et al.* (1992) showed that there was a significantly lower proportion of *streptococcus mutans* in the plaque adjacent to orthodontic brackets bonded with glass ionomer cement, in relation to the total viable count, compared with plaque adjacent to composite retained orthodontic brackets one month after commencing treatment (Hallgren *et al.*, 1992).

4. Salivary factors

Saliva is one of the most important factors that can affect the dynamics of mineral loss and gain at the enamel-plaque fluid interface. Salivary pH, flow rate and buffering capacity can influence the rate of progression of demineralization and the repair of the teeth (Newbrun, 1989; Higham, 2014). The risk of demineralization increases in patients in non-fluoridated communities compared with patients who live in areas with optimal fluoridation. An increase in the salivary fluoride reservoir is achieved via intake of fluoridated water throughout the day and via daily use of fluoride- containing dentifrice. Saliva is the vehicle which delivers fluoride ions to the enamel-plaque fluid interface (Higham, 2014).

Saliva acts as a buffer when the tooth surface is exposed to carbohydrate substrates and plaque acidity, and the microbial composition of dental plaque. During orthodontic treatment, the maxillary anterior teeth have high incidence of demineralization and this may be due to reduced saliva flow in that site (Gorelick *et al.*, 1982; Chang *et al.*, 1997; Aghoutan *et al.*, 2015). The labial surfaces of the anterior teeth are found to have a higher frequency of demineralization when compared with the lingual surface where there is more salivary exposure. This suggests that accessibility to saliva may be a major factor in preventing enamel demineralization (Aghoutan *et al.*, 2015).

Additionally, as the orthodontic bonding technique involves enamel etching, it can also partially lead to enamel demineralization due to decalcified surface of enamel created by acid etching (Hu & Featherstone, 2005). Although, composite resin cements are routinely used for bonding orthodontic brackets, it can produce enamel demineralization, enamel loss and a risk of enamel cracks during bonding. Additionally, the presence of flash around the bracket predisposes it to plaque accumulation, thus increasing the risk of demineralization of the surrounding enamel (Gorelick *et al.*, 1982; Toledano *et al.*, 2003).

Enamel demineralization or white spot lesions can also take place under the bracket surface, because of the polymerization shrinkage of the adhesive material which may promote micro-gap formation between the adhesive material and enamel surface, leading to micro-leakage. This would consequently allow microbial entrance and consequent enamel decalcification of the enamel surface (James *et al.*, 2003).

2.5.5. Prevention of enamel demineralization:

The prevention of enamel demineralization during orthodontic treatment has become a critical concern. An appropriate good oral hygiene regime that includes proper tooth brushing with fluoridated dentifrices and mouth washes to prevent white spot lesion in the orthodontic patient (Bishara *et al.,* 2008*)*. Additionally, enamel sealing and fluoride application is important to prevent extent of demineralization in the orthodontic patient. Because the mineral content of dental enamel is in equilibrium with its environment and saliva contains all the necessary elements for hydroxyapatite crystal growth superficial decalcification may disappear over time (Rossouw*,* 2010*)*.

In 1980, Menaker showed that fluoride ion inhibits the bacterial activity of *Streptococcus Mutans* by interfering with initial bacterial adhesion, thus affecting colonization and bacterial metabolism. In 1982, Maltz and Emilson showed that the inhibitory effect of fluoride on bacteria, decrease the pH and inhibits bacterial acid production and growth. Prevention therapies include oral hygiene maintenance, fluoride rinses, and topical fluoride applications; and a cementing agent that releases fluoride so as to inhibit demineralization and caries formation on tooth structure adjacent to the orthodontic bands or brackets (Bishara *et al.*, 1989: Paschos *et al.*, 2009). Other proposed benefits of using glass ionomer cements include: the release of fluoride over several months, which may contribute to the possible development of a modified, less cariogenic microflora (Matalon *et al*, 2005) and also because these cements do not need pre-treatment of the enamel with phosphoric acid to create conditions for mechanical bonding.

Benham *et al* (2009) showed that the use of highly filled (58%) pit and fissure sealant before bracket bonding provided a significant reduction in enamel demineralization during orthodontic treatment.

2.5.6. Measuring demineralization:

A variety of instruments have been used to evaluate enamel demineralization, including the hardness of mineralized tooth structure (Hodge, 1936 cited by De Marsillac *et al.*, 2008). Hardness is a characteristic of a material. It is defined as the resistance to indentation, and it is determined by measuring the permanent depth of the indentation (Poskus *et al.*, 2004).

Micro-hardness Test

The micro-hardness test term usually refers to static indentations made with loads not exceeding 1 kgf and is defined as the resistance to permanent deformation caused by indentation after load application (Poskus *et al.*, 2004). The micro-hardness is measured with the use of tests developed by Knoop and Vickers (Anusavice, 1996). It may be used to measure hardness of dental material and mineralized dental tissue. The procedure of the test is similar to the standard Vickers hardness test, except it is done on a microscopic scale with higher precision instruments. The Vickers method is the more commonly used micro-hardness test.

The Vickers hardness tests is done with the use of an elongated diamond pyramid-shaped point (indenter) that is pressed onto the test material under a well-defined load for a given time. Removal of the indenter results in an indent which resembles the shape of a pyramid-square shaped impression (Poskus *et al.*, 2004).

The size of the resulting indentation is determined with the aid of a microscope. The indenter causes a surface area deformation at the mineralized tissue under analysis. Hardness values are a measure of the mechanical resilience of the enamel due to the penetration of an indenter (De Marsillac *et al.*, 2008). The deeper the penetration of the indenter the softer is the enamel and the more the demineralization. Therefore, the level of enamel mineral content can be detected by micro-hardness evaluation as the mineral content affects the hardness of dental tissue (Torii *et al.*, 2001).

The followings are the most common hardness test methods used:

1. Rockwell hardness test.
2. Brinell hardness.
3. Vickers hardness.
4. Knoop hardness.

1. Rockwell Hardness Test

The Rockwell hardness test method is the most commonly used hardness test method (O'Brien, 2002). It consists of indenting the test material with a diamond cone or hardened steel ball indenter. It uses two loads, one applied directly after the other (Tabor, 1970). The first load, known as the "minor", load F0 usually 10 kilograms is applied to the specimen. This load represents the zero or reference position that breaks through the surface to reduce the effects of surface finish, and to help seat the indenter. The difference in the depth of the indentation between the minor and major loads provides the Rockwell hardness number (Rockwell Hardness Testing, 2010).

The benefits of the Rockwell hardness method include the direct Rockwell hardness number readout and rapid testing time, generally is easier to perform, and more accurate than other types of hardness testing methods (Rockwell Hardness Testing, 2010).

2. The Brinell hardness Test

The Brinell hardness test method is the best for achieving the bulk or macro-hardness of a material, particularly those materials with heterogeneous structures. It involves indenting the test material with a 10 mm diameter hardened steel or carbide ball (Tabor, 2000).

The Brinell ball makes the deepest and widest indentations compared to the other hardness test methods. So the test averages the hardness over a wider amount of material, giving a more accurate account for multiple grain structures and any irregularities in the uniformity of the material (Brinell hardness, 2010).

3. Vickers Hardness Test

The Vickers hardness test method, referred to as a micro-hardness test (Tabor, 1970). The Vickers hardness test was developed in 1921 by Robert L. Smith and George E. Sandland at Vickers Ltd as an alternative to the Brinell method to measure the hardness of materials.

The benefits of the Vickers hardness test include the ability to get extremely accurate readings, the use of one type of indenter for all types of metals and surface treatments and the ease of use compared to other hardness tests (Anusavice *et al*., 2012; Vickers Hardness, 2010). While the test indentation is very small in a Vickers test, it is useful for measuring individual microstructures (Anusavice *et al.*, 2012; Vickers Hardness, 2010).

Sample preparation is required with a Vickers hardness test to provide a small enough specimen that can fit into the tester and to ensure the sample can be held perpendicular to the indenter. Usually the prepared samples are mounted in a plastic medium to facilitate the preparation and testing (Vickers Hardness, 2010).

4. Knoop hardness test

The Knoop hardness test method, also referred to as a micro-hardness test method, was developed in 1939 by Knoop and colleagues at the National Bureau of Standards in the United States. The Knoop hardness indenter is a diamond ground to pyramidal form that produces a diamond shaped indentation having approximate ratio between long and short diagonals of 7:1. The depth of indentation is about 1/30 of its length (Tabor, 1970). The hardness of the material is determined by the depth to which the Knoop indenter penetrates (Anderson, 1976).

The Knoop indenter is different from the pyramid indenter that is used on a Vickers test which is more elongated or rectangular in shape (Anderson, 1976). It is suitable to use for measuring the hardness of brittle material such as tooth enamel (Anderson, 1976).

2.6 Conclusion:

From the literature review it appears that despite the use of different adhesive cements showing favourable properties there is still evidence of demineralisation and micro-leakage.

Around orthodontic brackets the use of resin modified glass ionomer cements significantly reduces enamel mineral loss due to its fluoride releasing properties compared with composite resins. However micro-leakage between the adhesive material and enamel surface are still a concern. Micro-leakage may lead to severe consequences such as enamel demineralization, enamel discoloration and failure of bond strength. It is also well known that micro-leakage and enamel demineralization increases the possibility of recurrent caries, post-operative sensitivity and is an aesthetic concern. Therefore the hypothesis of this study is that there is more enamel demineralization and less micro-leakage when composite resin adhesive are used compared to resin modified GIC.

Chapter 3

Aim and Objective

Chapter 3

Aim and Objective

3.1. Aim:

To assess micro-leakage and enamel demineralization (softening) around orthodontic direct attachment (brackets) using three different orthodontic cements.

3.2. Objectives:

- To determine the degree of micro-leakage and enamel demineralization (softening) around bonded orthodontic attachments using resin reinforced or modified GIC, other resin modified GIC and composite resin.

- To compare the micro-leakage and enamel demineralization (softening) pattern around bonded orthodontic attachments when using resin reinforced or modified GIC, other resin modified GIC and composite resin.

Chapter 4

Material and methodology

Chapter 4

Material and methodology

Introduction

An in-*vitro* study assessing and comparing micro-leakage and enamel demineralization using three different orthodontic cement Fuji Ortho LC, Rely X luting 2 and Transbond XT cements. The dye penetration technique was used to evaluate micro-leakage and micro-hardness testing was performed to assess enamel demineralization. The dye penetration technique is the most commonly used method in clinical and laboratorial studies to detect the micro-leakage in dental adhesive because of their susceptibility for bonding to tooth structure or restorative material, low cost and non-toxic properties (Going, 1972). Micro-hardness testing is widely used to study demineralization and/or remineralization in human teeth (Argenta *et al.,* 2003) or in bovine teeth (Argenta *et al.,* 2003). The accuracy and reproducibility of this test indicates that its applicability is very reliable for sound or demineralized hard dental tissues. Additionally, the operator error for hardness measurements has been found to be less than 5% (Purdell-Lewis *et al.,* 1976).

4. Materials and Methods:

4.1 Study sample:

Eighty one intact (non carious) premolars indicated for extraction, collected from patients (guardians) who consented to their use for the study were obtained from the Dental Faculty of the University of the Western Cape (Appendix). The extracted teeth were stored in distilled water at 4 degrees Celsius for 24 hours.

4.2 Selection Criteria

The teeth were screened under a 10x magnification mirror for any defects. Teeth that showed any of the following criteria were excluded from the study.

- anatomical defects
- caries
- restorations
- enamel cracks

4.3 Material used:

1. Extracted premolars.
2. Orthodontic Adhesives (Fuji Ortho LC (figure 4.1.), Rely X luting 2 cement (figure 4.2.), Transbond XT (figure 4.3.).
3. Brackets (Ormco Series 2000; first and second bicuspid with hook, part No. 303–1511, lot No. 50412821, Ormco, Orange, Calif)
4. Dye material (Methylene blue).

Figure 4.1. Fuji Ortho LC

Figure 4.2. Rely X luting 2

Figure 4.3. Transbond XT

a. Study Design

i. Micro-leakage sample:

Sixty (60) teeth divided into 3 groups of twenty teeth each, were used to test for micro-leakage. The groups were:

Group 1: Fuji Ortho LC

Group 2: Rely X luting 2

Group 3: Transbond XT

ii. Demineralization sample:

Twenty one (21) teeth divided into 3 groups of seven teeth each, were used to test for demineralization. The groups were:

Group 1: Fuji Ortho LC

Group 2: Rely X luting 2

Group 3: Transbond XT

b. Method

All the teeth in the study sample (81) were stored in distilled water at 4 ° Celsius.

i. Preparation of teeth

The teeth were removed from the refrigerator, cleaned with pumice to remove the debris, rinsed with distilled water and dried with compressed air. They were divided into three groups and the three different adhesives applied. Each of the adhesives was used according to the manufacturer's instructions. The bracket was selected and adapted optimally to the crown of each tooth. Twenty brackets were used for each of the micro-leakage test groups and seven brackets for each of the micro-hardness test groups.

ii.Bonding Procedure

Group 1: Fuji Ortho LC

Fuji Ortho LC (GC Fuji II LC GC Corporation Tokyo, Japan) was mixed according to manufactures instruction and applied directly to the fitting surface of each bracket (GAC Omni-Arch, GAC International). An explorer was used to position the bracket by firmly pressing it onto the tooth surface to ensure good bond strength and reduce sliding of the bracket. The excess cement was removed with an explorer. To set the bracket on the tooth surface, the material was light cured (quartz-tungsten-halogen (QTH) light cure unit) for 10 second per surface of the enamel according to the manufactures instruction.

Group 2: Rely X luting 2 cement

The desired amount of mixed Rely X luting 2 cement was dispensed onto a mixing pad. The dispensed Rely X luting 2 cement was mixed with a spatula for 20 seconds. A thin layer of cement was applied on the fitting surface of the orthodontic bracket, fitted onto the teeth and light cured (quartz-tungsten-halogen (QTH) light cure unit) for 5 seconds per surface of the enamel according to the manufacture. The excess cement was removed with an explorer after 2 minutes, when the cement became waxy.

Group 3: Transbond XT

Transbond XT adhesive cement unlike Fuji Ortho LC and Rely X luting 2 cement requires acid etching prior to bonding as per manufactures instructions.

Etching

Etching was performed with 37% phosphoric acid gel. It was carefully applied to the enamel surface of each tooth for 30 seconds, rinsed with a water spray for 20 seconds to remove the acid gel and dried with air until the etched enamel surface appeared chalky white as recommended by the manufacturer.

A light coat of Transbond XT primer was applied with a brush to the entire buccal surface. The brush was used to ensure that the application of the primer was of a uniform thickness and the primer was then exposed to a curing light for 15 seconds.

The Transbond XT cement was applied directly to the fitting surface of each bracket. The bracket containing the Transbond XT cement was pressed firmly onto the tooth surface to minimize resin excess, bracket drifting and maximize bond strength. Excess bonding material around the bracket was removed using an explorer before light curing (quartz-tungsten-halogen (QTH) light cure unit) for 20 seconds with a curing light per surface of the enamel according to the manufacture (figure 4.4.) and then the next step is varnishing.

Figure 4.4.QTH light cure unit

c. Micro-leakage assessment

i. Varnishing

After bonding all the teeth they were rinsed in tap water and air dried, and then coated with two layers of nail varnish (Charlie, Revlon, New York) up to 1 mm at the bracket margins to prevent dye penetration into the dentinal tubules or the lateral canals (Figure 4.5.) (Loguercio *et al*, 2004).

Figure 4.5. The teeth fitted with brackets coated with two layers of nail varnish

ii. Thermo-cycling

Following the application of the nail varnish, the teeth were placed in three separate porous bags according to the experimental groups. The porous bags were exposed to 500 thermo-cycles between 5°C and 55°C, with a dwell time of 15 seconds in a buffered (pH 7) 1% methylene blue solution dye (Figure 4.6.) (figure 4.7.) (Grobler *et* *al*., 2007; Loguercio *et al.*, 2004). The thermal cycling was done to simulate the temperature changes that take place in the oral cavity. All specimens were subjected to thermo-cycling according to the International Organization for Standardization (ISO) TR11405 standard.

Figure 4.6. Thermo-cycle

iii. Dye penetration

The Dye penetration technique was used for micro-leakage assessment. Superficial dye was removed with a brush, the nail varnish was removed with an acetone solution and all the teeth were again cleaned with water, dried and embedded in self-curing acrylic up to the occlusal surface of the direct attachment in a clear casting resin (Fibroglas®, SouthAfrica) and was allowed to harden (figure 4.8.) (Gillgrass *et al.,* 1999). They were then transferred and stored in specimen bottles containing distilled water at room temperature until the time of sectioning.

Figure 4.7. Teeth after thermo-cycle

Figure 4.8. Tooth embedded in self-cure acrylic for section

iv. Sectioning

All the teeth were sectioned into three parallel transverse sections. This was done with a low-speed 0.35 mm thick blade diamond saw disk cutter (Isomet, Buehler, and Lake Bluff, Ill) water cooled microtome (Struers Minitom, Germany) (figure 4.9.) (figure 4.10.) through the mesio-distal direction. The transverse sections were 400 microns in thickness.

Figure 4.9. Low speed saw

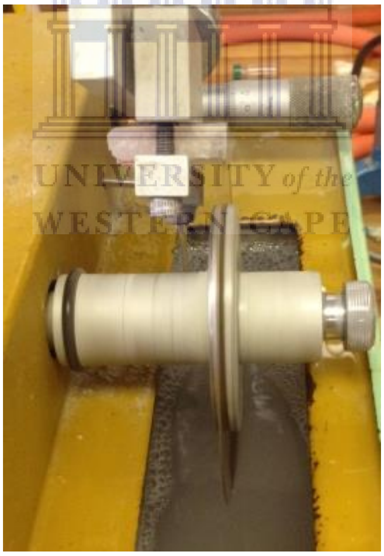


Figure 4.10. Tooth sectioning with diamond cutter

Three slices were obtained from each tooth one before the bracket, second one through the bracket and third one after the bracket (figure 4.11.). The transverse tooth slices were labelled and placed on the stereomicroscope.

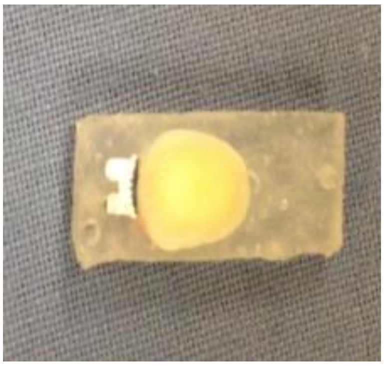


Figure 4.11. Cross section (cut through the bracket)

v. Microscopy and Scoring

The transverse tooth sections were viewed under a stereomicroscope (Nikon, Japan) at a 40x magnification (figure 4.12.). Photographs were taken with a Leica camera (Leica DFC 290 micro-systems, Germany) fitted onto the stereomicroscope. The ACDSee digital imaging software programme was used to transfer the photographs to a computer to measure the dye penetration along the enamel–adhesive and adhesive– bracket interfaces, both on the gingival and occlusal edge at 40 x magnification.

Figure 4.12. Stereomicroscope

Micro-leakage was determined by measuring the deepest dye penetration from the occlusal and gingival margins of the direct attachment at both the enamel-adhesive and adhesive-direct attachment interfaces with an electronic digital calliper (figure 4.13.). The nearest recording up to 0.5 mm was recorded as a micro-leakage value (Uysal *et al.,* 2010). An ordinal scale ranging from 0 to 3 was used to score the dye penetration (Arhun *et al.,* 2006). The total percentage of micro-leakage for enamel – adhesive and adhesive–bracket interfaces was obtained by summing the percentages of micro-leakage observed at the occlusal and gingival edges of each interface. The mean percentage of micro-leakage per tooth was calculated (Vicente *et al.,* 2009). The scoring criteria are summarized in Table 4.1 (Arhun *et al.,* 2006).

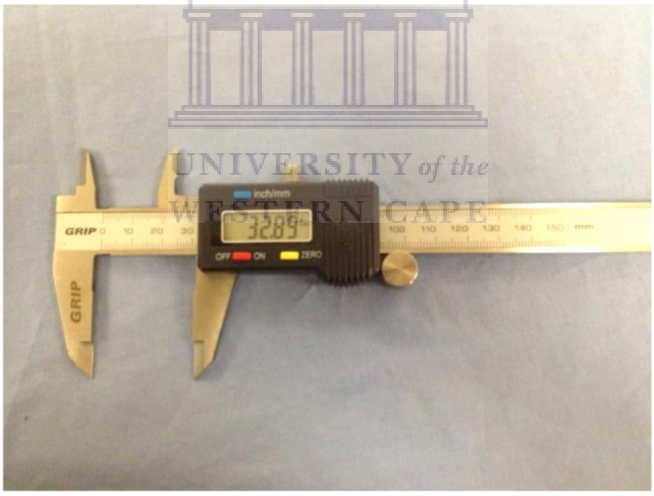


Figure 4.13. Electronic digital callipers

Table 4.1 Criteria for micro-leakage evaluation of dye penetration along the tooth – adhesive interface.

Score	Details
0	No dye penetration
1	Dye penetration restricted to 1mm of bracket-adhesive interface or adhesive-enamel interface
2	Dye penetration into the inner half (2mm) of bracket-adhesive or adhesive-enamel interface
3	Dye penetration into 3mm of bracket-adhesive or adhesive-enamel interface

d. Demineralization assessment:

i. Preparation of sample

The teeth were prepared by debriding the soft tissue remnants, cleaned and polished using prophylactic rubber cups with pumice slurry, thoroughly rinsed and dried. The roots of the teeth were sectioned below the cemento-enamel junction using a diamond disc.

ii. Mounting procedure (Amra *et al.,* 2007)

The teeth were embedded in self-curing acrylic resin (Orthocryl, Dentaurum) in PVC pipes with dimensions of 7 mm high, 20 mm outer diameter, and a wall thickness of 2 mm. The mid-buccal surfaces of the teeth; the areas ear-marked for the bonding of the orthodontic bracket; were arranged parallel to the outer rim of the PVC pipe. Care was taken to ensure that the tooth surface projected about 1mm above the rim of the pipe as the indenter can be worked easily. The PVC pipe supported crowns were then positioned on a table surface and the chemically cured acrylic resin poured around the tooth specimen. Care was taken to ensure that no resin contaminated the buccal enamel surface. The teeth were allowed to stand until polymerisation of the resin had occurred (figure 4.14.).

After completing the embedding, the enamel surfaces of all the teeth were ground using the Metaserv Universal Polisher (Surrey, England) (figure 4.15.). The enamel surfaces were sequentially ground under running water first with 100 grade, 400 grade and 800 grade carborundum papers to flatten the enamel buccal surface. Finally a 1000 grade carborundum paper was used to polish and smooth the enamel surface.

Figure 4.14. Teeth embedded in chemical cured acrylic

Figure 4.15. The Metaserv Universal Polisher

iii. Enamel micro-hardness tests for baseline values

A digital hardness tester with Vickers diamond indenter (Zwick RoellIndentec (ZHV; Indentec UK) (figure 4.16.) was used to measure surface micro-hardness of enamel before and after the bracket fitting. Firstly, the area on which the bracket was to be bonded on the enamel surface of the premolars was demarcated with a pencil by shading and this was used as a reference from which the different areas for indentations could be easily identified under the microscope (figure 4.17.)**.** Ten indentations were made on the shaded area of the enamel surface of each tooth with a 300g load applied for 15 seconds to establish the baseline hardness value. The indenter was always perpendicular to the enamel surface (at a primary magnification of 10x) (figure 4.18.). The hardness of the enamel within 50 µm in the shading area was determined before fitting the bracket on the tooth as (control group). This was done for the entire sample. The enamel micro-hardness values were recorded in a tabular form.

The teeth were subsequently bonded (figure 4.19.) as described earlier. This was followed by thermo-cycling in the same way as previously described to simulate the temperature changes that take place in the oral cavity.

Figure 4.16. Digital hardness tester

Figure 4.17. shading of the enamel surface

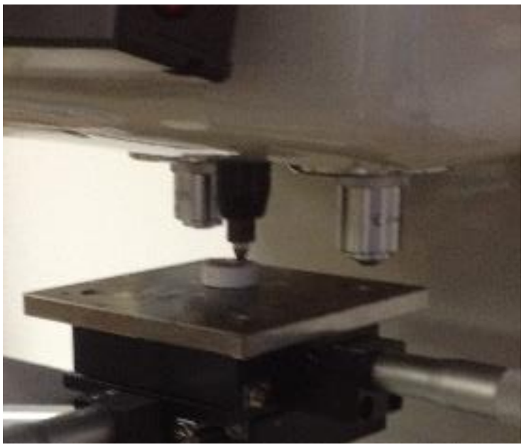


Figure 4.18. Indentations were made on this enamel surface

Figure 4.19. Bracket fitted on enamel surface

iv. Post demineralisation (softening) micro-hardness test

After the thermo-cycling process, de-bonding was carried out according to the method described by Årtun and Bergland (1984), using double bladed de-bonding pliers (figure 4.20.). Remnant cement was carefully removed with a scalpel blade. All the teeth were then viewed under the microscope to ascertain that all the cement was removed and no visible enamel damage was present.

To determine the degree of enamel demineralization as a result of the bracket placement enamel micro-hardness was measured again in the same way as previously described. Micro-hardness close (50µm) to the indents made (controls) was determined again after de-bonding the bracket. The results were recorded in an excel spreadsheet and submitted to the statistician for the analysis.

Figure 4.20. Double bladed de-bonding pliers

Chapter 5

Result

Chapter 5

Results

5.1 Micro-leakage

5.1.1 Micro-leakage at the occlusal and gingival margin

Micro-leakage scores determined by measuring the deepness of the dye penetration from the occlusal and gingival margins of the bracket at both the enamel-adhesive and adhesive-bracket interfaces using an electronic digital calliper were recorded and reflected in the tables below**.**

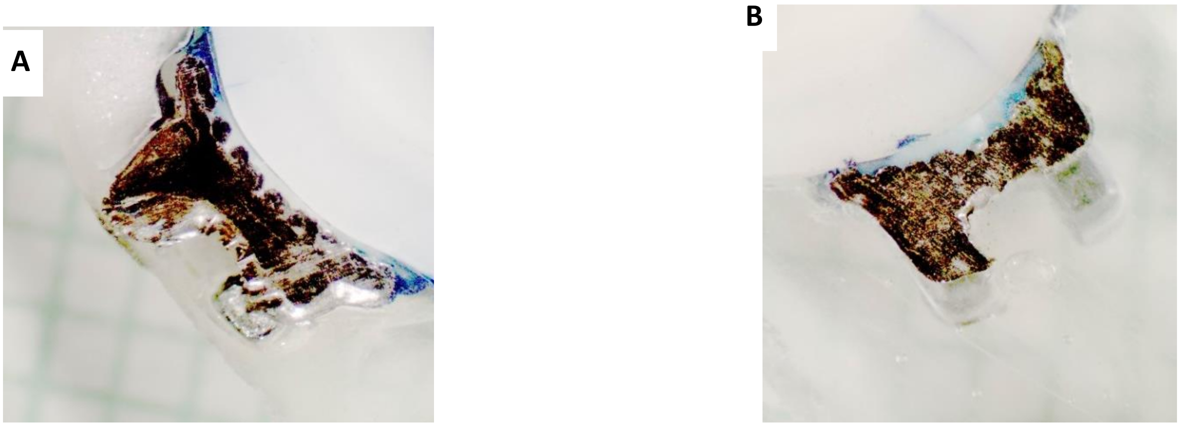
Table 5.1: Micro-leakage scores for individual teeth.

samples	**Group 1 (Fuji Ortho LC)**	**Group 2 (Rely X luting 2)**	**Group 3 (Transbond XT)**
1	1	3	1
2	2	3	0
3	0	3	0
4	3	2	0
5	3	3	0
6	2	3	0
7	0	3	0
8	2	3	0
9	3	3	0
10	3	3	0

11	2	3	1
12	0	3	1
13	2	3	0
14	1	3	1
15	1	3	0
16	2	3	0
17	3	3	0
18	1	3	0
19	2	3	0
20	2	3	1

Figure 5.1: Dye penetration pattern at the occlusal and gingival margins of the bracket at both the enamel-adhesive and adhesive-bracket interfaces.

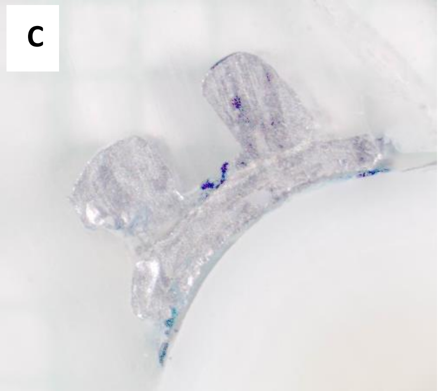
Group1 : Fuji Ortho LC (Resin reinforced GIC) :

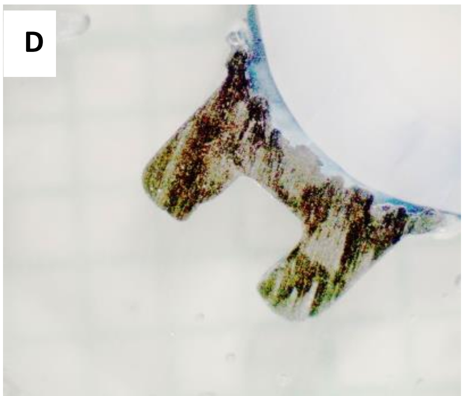


Occlusal margin

Gingival margin

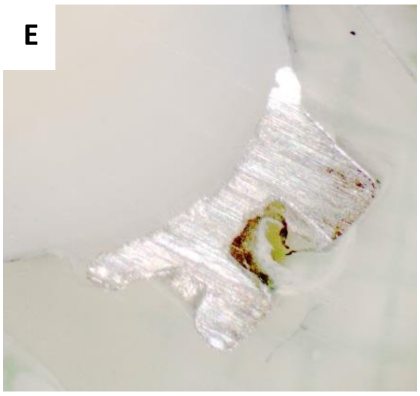
Group 2 : Rely X luting 2 (Resin modified GIC):





Occlusal margin Gingival margin

Group 3 : Transbond XT (conventional composite):



Occlusal surface Gingival surface

The above photographs (figure 5.1), illustrated the dye penetration pattern in each of the three groups. Dye penetration is very evident in the Fuji Ortho LC group at occlusal and gingival margin (Group 1A +1B) and on the gingival margin of the Rely X luting 2 group (Group 2D). There is also some evidence of dye penetration at the occlusal margin of the Rely X group (Group 2C). There is no evidence of dye penetration in the Transbond XT group (Group 3E +3F).

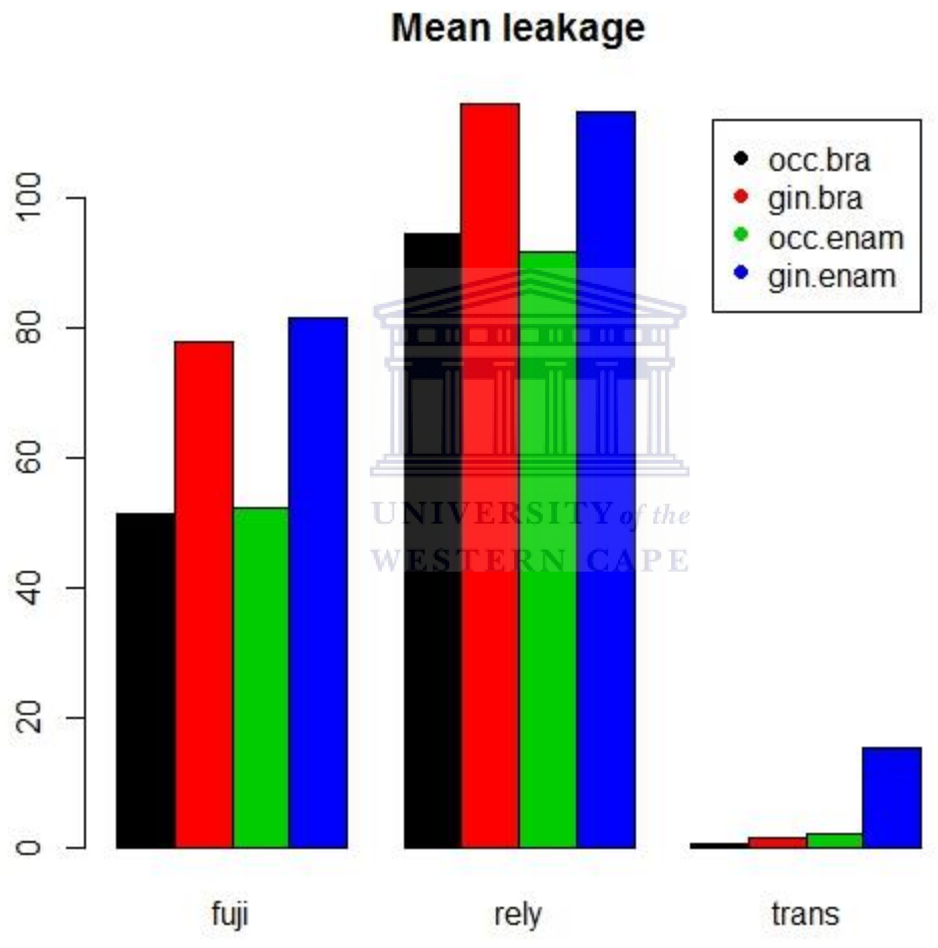
Table 5.2: The Mean Micro-leakage Score:

	Occlusal bracket-adhesive interface	Gingival bracket-adhesive interface	Occlusal enamel-adhesive interface	Gingival enamel-adhesive interface
Fuji Ortho LC	51.55	77.98	52.24	81.70
Rely X luting 2	94.42	114.49	91.67	113.17
Transbond XT	0.55	1.40	2.19	15.24

Table 5.2 shows the mean micro-leakage scores are higher on the side of the gingival margins than that of the occlusal margins.

Figure 5.2: Mean Micro-leakage at adhesive bracket interface and adhesive enamel interface on the occlusal and gingival margins.

Fuji Ortho LC = fuji . Rely X luting 2 = rely. Transbond XT = trans. Occlusal-bracket = occ.bra. Gingival-bracket = gin.bra. occlusal-enamel = occ.enam. Gingival-enamel = gin.enam.



The graphs (figure 5.2) illustrates that there was more micro-leakage at the gingival margins than on the occlusal margins. There was significantly more micro-leakage in the Rely X luting 2 sample (group2) when compared with the Transbond XT sample (group3).

Table 5.3: Comparison of total micro-leakage at the enamel–adhesive interface and bracket–adhesive interface;

Groups	Means	T(19) value	P value
Fuji ortho LC:			
bracket-adhesive interface	26.43	3.73	0.001
enamel-adhesive interface	29.46	4.22	0.0004
Rely X luting 2:			
bracket-adhesive interface	20.07	3.81	0.001
enamel-adhesive interface	21.50	3.83	0.0004
Transbond XT:			
bracket-adhesive interface	0.85	1,65	0.115
enamel-adhesive interface	13.05	2.50	0.022

The table above show the results of the paired t-tests comparing the occlusal and gingival means at the enamel–adhesive interface and bracket–adhesive interface. Every P-value but one, shows statistically significant differences. The rate of micro-leakage in gingival margins at enamel-adhesive interface was higher than bracket-adhesive interface.

The results for group 3 (Transbond XT) were confirmed by coding non-zero values as 1 and performing the Mcnemar tests.

5.1.2 Comparison of Micro-leakage between the three groups:

Due to the extremely skew distributions of micro-leakage with group3 Transbond XT (composite resin) the usual one way analysis of variance for group comparisons can give inaccurate P-values, therefore comparisons of groups Fuji Ortho LC (resin reinforced or modified GIC) and Rely X luting 2 (resin modified GIC) were done by two-sample t-tests.

Table 5.4: Comparisons of the micro-leakage between Fuji Ortho LC and Rely X

	t(38) value	P value
Occlusal bracket-adhesive	3.48	0.001
Occlusal enamel-adhesive	3.38	0.002
Gingival bracket-adhesive	3.24	0.003
Gingival enamel-adhesive	2.65	0.012

The table above shows all four of the tested differences are statistically significant.

The differences between Transbond XT (composite resin) and the other two groups are so obvious as hardly to require formal testing. One way of performing valid tests is to transform all non-zero value observations to 1 and then to compare the proportions of zeroes in the groups as is tabulated in table 5.5.

Table 5.5: The observed chi-squared tests are:

	chi-squared test	P value
Occlusal bracket-adhesive	51.45	<0.001
occlusal enamel-adhesive	47.29	<0.001
gingival bracket-adhesive	47.44	<0.001
gingival enamel-adhesive	26.94	<0.001

The table above shows the chi-squared test of equality of proportions can be applied. Its result is a chi-squared statistic, on 2 degrees of freedom, of which a value exceeding 13.82 indicates significance at level <0.001. The result showed statistically significantly lower levels of micro-leakage for Transbond XT (P= <0.001).

5.2 Demineralization

The tables and graphs below illustrate the amount of demineralization recorded in Vickers hardness before and after the bracket fitting.

5.2.1 Comparison of demineralization before and after bonding:

Table 5.6: Vickers Hardness Value (VHV) of each tooth before and after bonding (mean values):

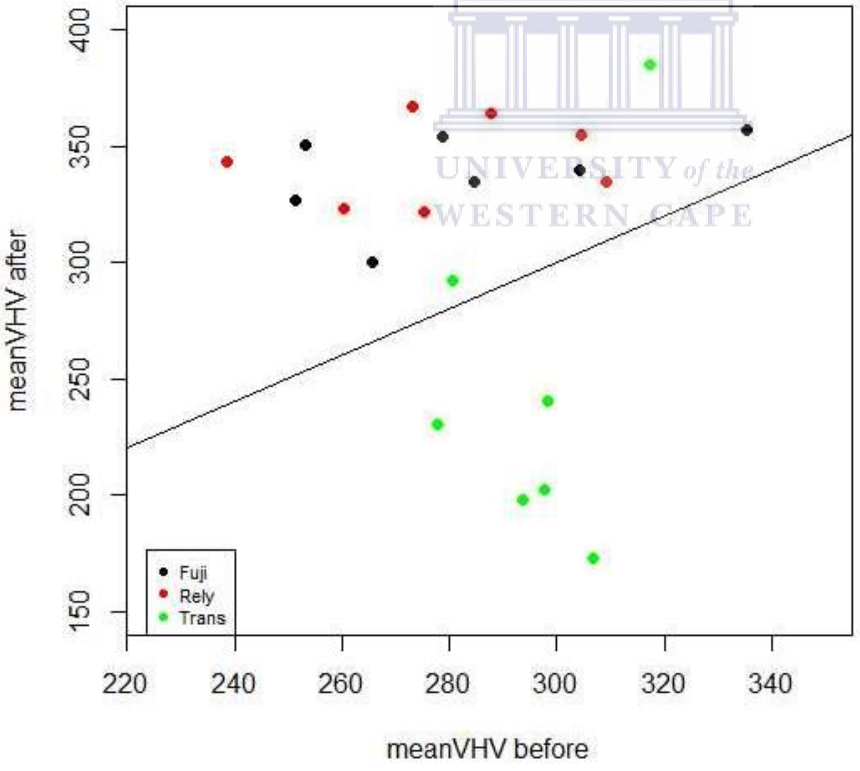
Fuji Ortho LC		Rely X luting 2		Transbond XT	
Before bonding	After bonding	Before bonding	After bonding	Before bonding	After bonding
304.3	339.6	309.3	334.5	298.4	240.1
335.4	356.9	238.6	343	317.4	384.9
284.7	334.5	273	367.1	293.7	197.9

265.8	300.4	275.4	321.9	297.7	202.2
253.3	350.7	287.9	364.4	280.5	291.9
278.8	354.4	304.7	354.7	306.7	173
251.3	326.8	260.3	323.3	277.8	230.3

The table above shows that the mean hardness value was higher for after bonding compared with it before bonding in the Fuji Ortho LC and Rely X luting 2 group. However, the Transbond XT, the mean hardness value was higher before bonding.

The mean hardness values of the three groups are illustrated graphically in figure 5.3.

Figure 5.3: The mean hardness values of the three groups*:*



The mean hardness values after bonding are plotted against their matching values before bonding. The straight line in the figure is a line through the origin with x where slope=1, i.e. points on the line would have equal before and after bracket bonding values. Fuji Ortho LC (resin reinforced or modified GIC), Rely X (resin modified GIC) points lie above the line, indicating significantly greater hardness. The Transbond XT (composite resin) points are much more widely scattered, two of them above the line and five below, which indicates no statistically significant change.

Table 5.7: The standard deviations (SD) of the mean VHV of the three groups:

Treatment	Fuji Ortho LC	Rely X luting 2	Transbond XT
after	19.75	18.58	72.16
Before	29.99	24.79	13.89

The table above show the Standard deviations of mean VHV data of the three groups show variability. Transbond XT has the highest standard deviation of the mean VHV compared to Fuji Ortho LC and Rely X luting 2 after bonding. The variability in the before bonding data is much the same within the three groups. Bartlett tests of homogeneity of variances were performed with the before and after bonding data. The result for before bonding is Chi-squared = 3.051, df = 2, p-value = 0.218, i.e. the variances are not significantly different. The result with after bonding data is Chi-squared = 13.435, df = 2, p-value = 0.001, which is statistically significant clearly indicating that Transbond XT has greater demineralization (softening) and less hardness than the Fuji Ortho LC and Rely X luting 2.

5.2.2 Comparisons of enamel demineralization in the three groups after bonding:

Table 5.8: The standard deviations (SD) of the three groups:

Groups	Before bonding		After bonding	
	Mean	SD	Mean	SD
Fuji Ortho LC	281.94	29.99	337.61	19.75
Rely X luting 2	278.46	24.79	344.13	18.58
Transbond XT	296.03	13.89	245.76	72.16

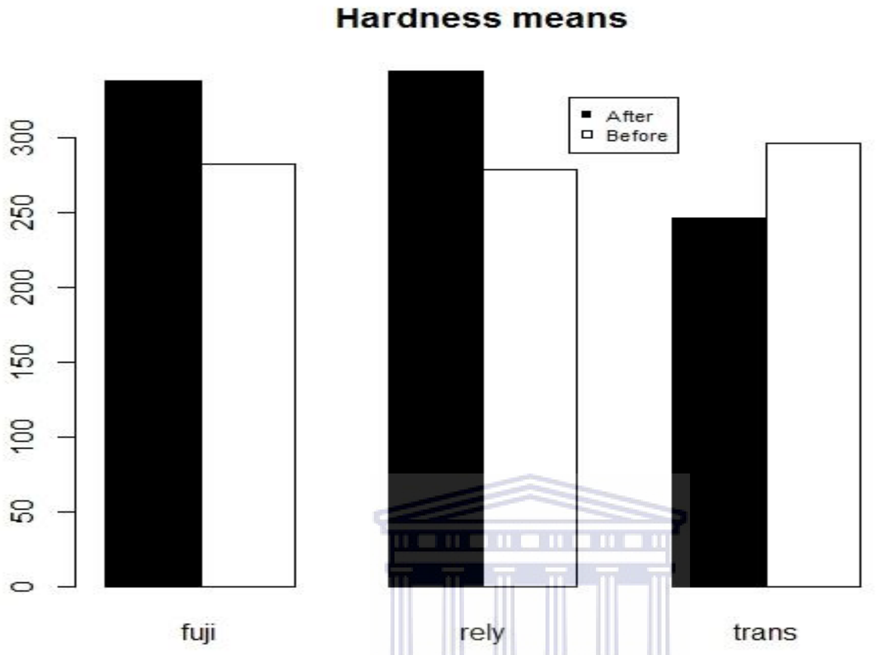
The table above shows that in the after bonding data the SD of Transbond XT is significantly greater than the other two SD's, so the Welch version of the usual analysis of variance test was used to obtain the result: F(2,11)=5.749, P=0.020, indicating significant differences between the mean. Two sample t-tests show that the Fuji Ortho LC and Rely X luting 2 mean are not significantly different from each other; t = -0.636, df = 12, p-value = 0.537. The Transbond XT mean differs significantly from both of the other two mean:

Transbond XT vs Fuji Ortho LC: t = 3.249, df = 6.9, p-value = 0.014(Welch t-test).

Transbond XT vs Rely X luting 2: t = 3.493, df = 6.8, p-value = 0.011(Welch t-test).

Figure 5.4: Hardness means of the teeth before and after bonding

Fuji Ortho LC = fuji. Rely X luting 2 = rely. Transbond XT = trans.



The figure above show the enamel was less demineralized in Fuji Ortho LC (group 1) and Rely X luting 2 (Group 2) than Transbond XT (group 3) after bonding. There was also no difference in the enamel micro-hardness before bonding. After bonding Transbond XT (group 3) differed significantly from Fuji Ortho LC (groups 1) and Rely X luting 2 (group 2) in the hardness. However, there was no significant difference between Fuji Ortho LC (groups 1) and Rely X luting 2 (group 2) at this level of confidence.

5.3 Conclusion:

Micro-leakage and enamel demineralization during orthodontic treatment is a significant clinical problem, and based on the results of this *in vitro* study, it can be concluded that Transbond XT had significant lower micro-leakage compared with Fuji

Ortho LC and Rely X luting 2. The enamel micro-hardness was measured to determine the degree of enamel demineralization (softening) showing Transbond XT had less hardness and greater demineralization than the Fuji Ortho LC and Rely X luting 2.

Chapter 6

Discussion

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Discussion

6.1 Adhesive cements:

New orthodontic cements, adhesive resins, and hybrid cement resin combinations offer improved physical properties, low solubility in oral fluids and clinical benefits, but there are clear differences in the clinical indications and contraindications for each class of cement (Ewoldsen & Demke, 2001). In an attempt with an understanding of the features, benefits, and limitations of adhesive cement, that can choose the adhesive cement accurately to obtain the optimal results.

The present study was undertaken to compare the micro-leakage and enamel demineralization pattern around bonded orthodontic attachments when using Fuji LC Ortho (resin reinforced or modified GIC), Rely X luting 2 (resin modified GIC) and Transbond XT (composite resin).

The application of glass ionomer cements provides greater fluoride release and adequate bond strength, (Rix *et al*., 2001). However, glass ionomer cements have shown greater bond failure rates than composite resins (Miller *et al.,* 1996). Resin modified glass ionomer cements have some characteristics that make them very desirable for orthodontic bonding like fluoride release properties as well as capability of providing satisfactory bond strength to enamel while bonding is performed in presence of moisture. In addition to micromechanical lock with enamel surface irregularities they provide chemical bonding resulting in superior bonding strength (Patil *et al.,* 2014).

Composite resins are one of the most commonly used adhesives in orthodontic bonding as they provide sufficient bonding strength and are easy to handle. However, they require a dry field and the amount of fluoride release is not sufficient for demineralization protection (Patil *et al.,* 2014).

6.2 Micro-leakage:

The micro-leakage under the orthodontic brackets was determined by the dye penetration method and is one of the most common methods (Taylor & Lynch, 1992; Vicente *et al.*, 2009; Gillgrass *et al.*, 1999; Arhun *et al.,* 2006; Choi *et al.*, 2000; Ramoglu *et al*., 2009; Ulker *et al*., 2009; Uysal *et al*., 2008; Uysal *et al*., 2010). The dye penetration method is a simple, relatively cheap and accurate (Uysal *et al.,* 2010). The dye penetration method involves exposure of the study sample to methylene blue, for a determined period (24 hour) and then viewing cross sections under a light microscope to determine the extent of leakage around the enamel adhesive interface and adhesive bracket interface (Uysal *et al.,* 2010).

In this study, micro-leakage evaluation was performed for three adhesive cements from two interfaces: bracket-adhesive and enamel-adhesive in both occlusal and gingival side. As well found dissimilar micro-leakage scores at occlusal and gingival side for all specimens at both the bracket-adhesive and enamel-adhesive interface. The rate of micro-leakage in the occlusal and gingival margins was significantly different and was higher in the gingival portion (every P-value, i.e. <0.05 indicating statistically significant differences).

A study by Arhun *et al.* (2006) showed that micro-leakage scores at the gingival side is greater than at occlusal side, and was significantly different and they concluded that the difference is due to the curvature of the tooth anatomy, which may result in relatively thicker composite at the gingival margin. Uysal *et al.* (2008) and Ulker *et al.* (2009) also obtained similar results as Arhun *et al*. (2006) but argued that low or no micro-leakage scores at occlusal side compared with gingival side, is due to the curing method, as the curing light was applied from the occlusal direction.

The differences between Transbond XT (composite resin) and the other two groups are so observable. The result showed statistically significantly lower levels of micro-leakage for Transbond XT (P= <0.001).

The most common cause of micro-leakage is polymerization shrinkage of composite resin and it varies by composition of adhesive as percentage of filler, the diluents or percentage of monomer conversion in that resin (Burgess *et al.,* 1999). The curing composites undergo polymerization shrinkage and subsequently micro-leakage occurs in restorative dentistry and orthodontic dentistry (Ferracane & Mittchem, 2003) (Arhun *et al.,* 2006). However the polymerization shrinkage and micro-leakage was less in orthodontic dentistry compared to restorative dentistry (James *et al*., 2003) Less micro-leakage in orthodontic dentistry is due to the application of a thinner layer of the composite resin and the shrinkage can pull the bracket closer to the enamel (Arhun *et al.,* 2006).

6.3 **Demineralization**:

Enamel demineralization is an unaesthetic issue that can occur during or after orthodontic treatment. In this study demineralization, which is a loss of mineral, was measured by evaluating the surface micro-hardness of enamel before and after the orthodontic direct attachment (bracket) fitting. The choice of this method for demineralization measurement depends on study procedure and the abilities of laboratory (White *et al*., 1992).

The degree of enamel demineralization as a result of the bracket placement was measured by the digital hardness tester, the Vickers diamond indenter (Zwick Roell Indentec (ZHV; Indentec UK). Removal of the indenter results in an indent which resembles the shape of a pyramid-square shaped impression which is microscopic (Poskus *et al.,* 2004).

The result of this study provided a comparison between the Fuji Ortho LC group, Rely X luting 2 group and Transbond XT group. The variability in the before bonding data is much the same within the three groups. The result with after bonding data is Chi-squared = 13.435, df = 2, p-value = 0.001, which is statistically significant clearly indicating that Transbond XT has greater enamel demineralization (softening) and less hardness than the Fuji Ortho LC and Rely X luting 2. The amount of enamel demineralization around and under the brackets more when using Transbond XT may be due to the acid etching technique and no fluoride content. The sequence of acid etching technique and bonding procedure of bracket to the enamel surface affects the balance of mineral loss and repair which leads to demineralization during orthodontic treatment (Chang *et al.,* 1997; Nkosi *et al*., 2008). On the contrary, Fuji Ortho LC and Rely X luting 2 did not show demineralization and had an increase in hardness of enamel after the bracket fitting. This may have been as a result of fluoride release which can be absorbed onto the surface of enamel crystals and inhibit dissolution, encourage remineralisation of porous enamel, decrease the solubility of enamel, decrease the dissolution rate in demineralization rate in acidic condition, thus inhibiting caries formation (Featherstone, 1999; Uysal *et al.*, 2010; Paschos *et al.*, 2009). Both Fuji Ortho LC and Rely X luting 2 presented with similar hardness levels as there was no significant difference between Fuji Ortho LC and Rely X luting 2 groups. In addition, resin modified glass ionomer cements such as Fuji Ortho LC and Rely X luting 2 adhesive cements also have antibacterial agent that inhibit caries formation especially along the enamel margins (Øgaard *et al.,* 2001). The combination of fluoride and antibacterial agent in the Fuji Ortho LC and Rely X luting 2 adhesive cements decrease white spot formation during orthodontic treatment (Arhun *et al.,* 2006).

As a result, consistent with the literature, teeth bonded with resin modified glass ionomer cement had significantly reduced lesion depth of enamel and mineral loss when compared with the composite resin cement (Vorhies *et al.,* 1998). Paschos *et al*. (2009) also showed that the use of Fuji Ortho LC has a significantly reduced lesion depth and less mineral loss compared with the other adhesive cements.

Thus the general use of adhesive materials that release fluoride has been recommended for orthodontic practice (Cohen *et al.,* 2003) to decrease the risk of demineralization (Uysal *et al.,* 2010). It is especially recommended for orthodontic patients who have compromised oral hygiene practices (Wilson & Donly, 2001). This study clearly shows that both Fuji Ortho LC and Rely X luting 2 cements have the ability to inhibit the demineralization of enamel around and under the bracket. Furthermore, Wilson & Donly (2001) observed more fluoride release from glass ionomer cement in-vitro studies when compared with in-vivo studies (Wilson & Donly, 2001).

In order to select the proper orthodontic bonding agent it is important to consider not only the amount of fluoride release of the RMGIC but also other factors such as bond strength, ease of handling of the cement during bracket placement (Wilson & Donly, 2001) and the amount of micro-leakage. So Transbond XT is currently the most preferred bonding adhesive cement in orthodontic practice (Wilson & Donly, 2001).

Chapter 7

Limitation

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Limitation

7 Limitations:

- Micro leakage and demineralization studies are extremely difficult to perform *in vivo* and results are only obtained in an *in vitro* setting by the use of extracted human teeth (Rueggeberg, 1991). *An in vitro* study, however, is observed as a prediction of what may actually happen in the clinical situation (*in vivo*).
- The main limitation of this study was related to the prediction of the clinical situation by the simulation of temperature changes in the mouth using thermo-cycling process. The thermo-cycling process may lead to unequal volume changes because of the linear thermal expansion of tooth structure and adhesives bonding differences. Differential thermal changes can induce mechanical stresses which lead to an increase in crack formation through the bonded interface.
- Factors that also influence the outcome of the results in micro-leakage studies are the media used, the storage time and the temperature at which the specimens are stored. In studies the time factor after extraction of the human teeth has not been specified (Hilton, 2002). The most common words used in the studies were either “freshly extracted” that used to describe sample collection but it seems difficult to extrapolate the exact time period (Hilton, 2002). In addition there is a variety of medium solutions used for the storage of extracted teeth used in micro-leakage studies and they are formalin, thymol,

chloramines, sodium azide, saline and water. These media may have different effects on enamel and dentine.

Chapter 8

Conclusion and Recommendation

Chapter 8

Conclusion and Recommendation

8.1 Conclusion:

Within the limitations of this in-vitro study, the following conclusions were reached:

1. Bracket cemented with Rely X luting 2 (groups 2) had significantly higher scores of micro-leakage at the enamel-adhesive and adhesive-bracket interfaces compared with Fuji Ortho LC (group 1) and Transbond XT (groups 3).
2. The gingival margins in all groups show higher micro-leakage scores than occlusal margins for both the enamel-adhesive and adhesive-bracket interfaces.

3. There was no difference in the enamel micro-hardness in before bonding the bracket with Fuji Ortho LC (resin reinforced or modified GIC) and Rely X luting 2 (RMGIC) and Transbond XT (composite resin), the after bonding mean hardness value was higher than the before bonding in Fuji Ortho LC (resin reinforced or modified GIC) and Rely X luting 2. However, in The Transbond XT the mean hardness value was higher before than after bonding.
4. Transbond XT statistically significant has less hardness and greater demineralization than the Fuji Ortho LC and Rely X luting 2.
5. The hardness of the enamel after bonding the bracket with Transbond XT (group 3) differed significantly from Fuji Ortho LC (groups 1) and Rely X luting

2 (group 2). However, there was no statistically significant difference between Fuji Ortho LC (groups 1) and Rely X luting 2 (group 2) at this level of confidence.

8.2 Recommendation:

With regard to the limitation of the study, the following recommendation can be considered for future research in the field of micro-leakage and demineralization.

Further in vivo research should focus on fluoride release and bond strength of adhesive materials in longitudinal study.

The three adhesive cements should also be experimented with hypo-mineralized enamel; this will provide a comparison regarding the optimal adhesive that should be used to improve the bonding of the bracket to hypo-mineralized teeth.

Chapter 9

References

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References

Abdelnaby, Y.L., Al-Wakeel, E.E., 2010. Influence of modifying the resin coat application protocol on bond strength and micro-leakage of metal orthodontic brackets. *Angle Orthod*, 80,378–384.

Argenta, R.M., Tabchoury, C.P., Cury, J.A., 2003. A modified pH-cycling model to evaluate fluoride effect on enamel demineralization. *Pesqui Odontol Bras*, 17, 241-246.

Agosta, C., Estafan, D., 2003. Eliminating micro-leakage from the composite resin system. *Gen,* 506-509.

Alani, A.H., 1990. The influence of dentin bonding agents upon the micro-leakage associated with composite resin restorations and their effects upon the dental pulp PhD Thesis, University of Dundee, Scotland.

Alani, A.H. and Toh, C.G., 1997. Detection of micro-leakage around dental restorations: a review. *Oper Dent*, *22*(4), 173-85.

Al-Ehaideb, A.A., Mohammed, H., 2001. Micro-leakage of “One Bottle” dentin adhesives. *Oper Dent,* 26,172-175.

Amra, I., Samsodien, G., Shaikh, A., Lalloo, R., 2007. Xeno III self-etching adhesive in orthodontic bonding: The next generation. *Am J Orthod and Dentofac Orthop*, 131, 160.e11-160.e15.

Anusavice, K.J., 1996. Phillips Science of Dental Materials. *10thedition, WB Saunders,* Chapter, 12, 273-284.

Anusavice, K.J., Shen*, C*., Rawls*,* H.R., 2012. Phillips Science of Dental Materials. *Elsevier,* 12, 63-65*.*

Anderson, J.N., 1976. Applied Dental Materials. *Blackwell scientific publications*, 5, 27-29.

Aghoutan, H., Alami, S., El Quars, F., Diouny, S., Bourzgui, F., 2015. White Spots Lesions in Orthodontic Treatment and Fluoride — Clinical Evidence. *Emerging Trends in Oral Health Sciences and Dentistry,* 10,317-339.

Arhun, N., Arman, A., Cehreli, S., Arikan, S., Karabulut, E., Gulsahi, K., 2006. Micro-Leakage beneath ceramic and metal brackets bonded with a conventional and an antibacterial adhesive system. *Angle Orthod,* 76, 1028-1034.

Arikan, S., Arhun, N., Arman, A., Cehreli, S.B., 2006. Micro-leakage beneath ceramic and metal brackets photopolymerized with LED or conventional light curing units. *Angle Orthod,* 76, 1035-1040.

Armstrong, W.D., Simon, W.J., 1951. Penetration of radiocalcium at the margins of filling materials: a preliminary report. *Journal of the American Dental Association,* 43, 684-686.

Artun, J., Bergland, S., 1984. Clinical trials with crystal growth conditioning as an alternative to acid-etch enamel pre-treatment. *Am J Orthod Dentofacial Orthop*, 85,333-340.

Årtun, J., Brobakken, B.0., 1986. Prevalence of carious white spots after orthodontic treatment with multibonded appliances. *Eur J Orthod,* 8, 229-234.

Atkinson, A.S., Pearson, G.J., 1985. The evolution of glass ionomer cements. *British Dental Journal* 159, 335-337.

Bakopoulou, A., Mourelatos, D., Tsiftsoglou, A.S., Giassin, N.P., Mioglou, E., Garefis, P., 2009. Genotoxic and cytotoxic effects of different types of dental cement on normal cultured human lymphocytes. *Mutat Res*, 672, 103–112.

Banks, P.A., Chadwick, S.M., Asher-McDade, C., Wright, J.L., 2000. Fluoride-releasing elastomerics-a prospective controlled clinical trial. *Eur J Orthod*, 22, 401-407.

Barber, D., Lyell, J., Massler, M., 1964. Effectiveness of copal resin varnish under amalgam restorations. *Journal of Prosthetic Dentistry,* 14, 533-536.

Barnes, I.E., 1977. The adaptation of composite resins to tooth structure Parts 1-4. *British Dental Journal,* 142, 122- 129, 185-191, 253-259, 319-326

Bassham, D.A., 1999. Measuring fluoridated and non-fluoridated sealant fluoride concentrations and their effect on Streptococcus mutans: An *in-vitro* Study. *Unpublished master's thesis West Virginia University,* 1-159.

Baumgartner, W.J., Bustard, R.E., Feierabend, R.F., 1963. Marginal leakage of amalgam restorations. *Journal of Prosthetic Dentistry,* 13, 346-353.

Behnan, S.M., Arruda, A.O., Gonza´lez-Cabezas, C., Sohn, W., Peters, M.C 2010. In-vitro evaluation of various treatments to prevent demineralization next to orthodontic brackets. *Am J Orthod Dentofacial Orthop, 138,712–713*.

Benham, A.W., Campbell, P.M., Buschang, P.H., 2009. Effectiveness of pit and fissure sealants in reducing white spot lesions during orthodontic treatment: a pilot study. *Angle Orthod*, 79,337-344.

Benson, P.E., Parkin, N., Millett, D.T., Dyer, F.E., Vine, S., Shah, A., 2004. Fluorides for the prevention of white spots on teeth during fixed brace treatment (Cochrane Review).In the Cochrane Library. Chichester: John Wiley, Issue 3

Benson, P.E., Shah, A.A., Millett, D.T., Dyer, F., Parkin, N., Vine, R.S., 2005. Fluorides, orthodontics and demineralization: a systematic review. *Journal of Orthodontics*, 32, 102–114.

Bernstein, L., 1965. Methods and factors involved in bonding orthodontic attachments to enamel. *Journal of Nihon University School of Dentistry*, 7, 96-102.

Bishara, S.E., Chan, D.C.N., Abadir, E.A., 1989. The effect on the bonding strength of fluoride application after etching. *Am J Orthod Dentofac Orthop,* 95,259-260.

Bishara, S., Laffoon, J., VonWald, L., Warren, J., 2001. Evaluation of a non-rinse conditioning solution and a compomer as an alternative method of bonding orthodontic bracket. *The Angle Orthodontist*, 71, 46– 465.

Bishara, S., Ajlouni, R., Laffoon, J.F., 2003. Effect of thermos-cycling on the shear bond strength of a cyanocrylate orthodontic adhesive*. American Journal of Orthodontics and Dentofacial Orthopedics*, 123, 21– 22.

Bishara, S.E., Oonsombat, C., Soliman, M.M.,Warren, J., 2005. Effects of using a new protective sealant on the bond strength of orthodontic brackets. *Angle Orthod*, 75,243-246.

Bishara, S.E., Ostby, A.W., 2008. White spot lesions: formation, prevention, and treatment. In *Seminars in Orthodontics*, 14(3), 174-182.

Bourke, A.M., Walls, A.W., McCabe, J.F., 1992. Light-activated glass polyalkenoate (ionomer) cements: the setting reaction *Journal of Dentistry,* 20**,** 115–120.

Boston, D.W., Jefferies, S.R., 2009. Effects of a 36% tooth bleaching gel on zinc phosphate cement. *J. Biomed. Mater. Res. Part B: Appl. Biomater*, 92, 456.

Boyde, A., Knight, P.J., 1969. The use of scanning electron microscopy in clinical dental research. *British Dental Journal,* 127, 313-322.

Boyd, R.L., 2001. Periodontal considerations during Orthodontic treatment. *In Bishara SE (Editor).Textbook of Orthodontics. WB Saunders.* Philadelphia, 442-453.

Boyles, G.A., 2007. Effects of fluoride varnishes and adhesives on bond strength and preventing enamel decalcification around orthodontic appliances: An *in-vitro* and *in vivo* Study. Unpublished master's thesis, West Virginia University, 1-68.

Brannstrom, M., Soremark, R., 1962. Penetration of Na22 ions around amalgam restorations with and without cavity varnish. *Odontologisk Revy,* 13, 331-336.

Brinell hardness Testing, 2010*.* http://hardnesstesters.com/Applications/Brinell-Hardness-Testing.aspx*. Retrieved on 11th July, 2016.*

Bowen, R.L., 1956. Use of epoxy resins in restorative materials. *J Dent Res*, 35,360-369.

Buonocore, M.G., 1955. A simple method of increasing the adhesion of acrylic filling materials to enamel surface. *Journal of Dental Research,* 34, 849-853.

Burgess, J.O., De Goes, M., Walker, R., Ripps, A.H., 1999. An evaluation of four light-curing units comparing soft and hard curing. *Pract Periodontics AesthetDent*, 11,125–132.

Cantwell, J.C., Cantwell, K.L., Claycomb, C.K., Gatewood, D.C., 1959. Study of marginal seal of dental restorations using C 14 labelled fructose. Abstract presented at the 37th general meeting *Journal of Dental Research* 38 *Abstracts of Papers* p755.

Cenci, M.S., Demarco, F.F., Carvalho, R.M., 2005. Class II composite resin restorations with two polymerization techniques: relationship between micro-tensile bond strength and marginal leakage. *J Dent,* 33,603-610.

Chadwick, L.B., Roy, J., Knex, J., Treasure, T.E., 2005. The effect of topical fluorides on decalcifications of patients with fixed orthodontic appliance: a systemic review. *Am J Orthod Dentofacial Orthop,* 128,301–306.

Chan, K.C., Swift, E.J., 1991. Leakage of a light-activated base: effect of dentin bonding agents*. J Prosthet Dent,* 65, 790-792*.*

Chang H.S, Walsh L.J, Freer T.J, 1997. Enamel demineralization during orthodontic treatment. Aetiology and prevention. *Australian Dental Journal,* 42 (5), 322-327.

Chatterjee, R., Kleinberg, I., 1979. Effect of orthodontic band placement on the chemical composition of human incisor plaque. *Arch Oral Biol,* 24, 97-100.

Chen, H.Y., Manhart, J., Hickel, R., Kunzelmann, K.H., 2001. Polymerization contraction stresses in light-cured packable composite resins. *Dental Materials,* 17, 253-259.

Choi, K.K., Condon, J.R., Ferracane, J.L., 2000. The effects of adhesive thickness on polymerization contraction stress of composite. *J Dent Res,* 79, 812–817.

Chung, C., Cuozzo, P.T., Mante, F.K., 1999. Shear bond strength of a resin reinforced glass ionomer cement: an *in-vitro* comparative study. *Am J OrthodDentofacOrthop,* 115, 52-54.

Christen, A.G., Mitchell, D.F., 1966. A fluorescent dye method for demonstrating leakage around dental restorations. *J. Dent. Res*, 45(2), 1.485-1.492.

Cohen, W.J., Wiltshire, W.A., Dawes, C., Lavelle, C.L.B., 2003. Long-term in vitro fluoride release and rerelease from orthodontic bonding materials containing fluoride. *Am J Orthod Dentofacial Orthop*, 124,571-576.

Creo, A.L., Mitra, S.B, Yates, R.M., 1989. Vitrabond light cure glass ionomer liner/base, Product Profile. *3M Dental Products Division, St Paul, Minn*.

Crim, G.A., 1987. Evaluation of a dental adhesive and cavosurface angle in preventing marginal leakage *New York State Dental Journal,* 53, 22-23.

Crim, G.A., Esposito, C.J.J.r., Chapman, K.W., 1985. Micro-leakage with a dentine-bonding agent. *General Dentistry,* 33, 232-234.

Davari, A., Yassaei, S., Karandish, M., Zarghami, F., 2012. In vitro Evaluation of microleakage under ceramic and metal brackets bonde with LED and plasma Arc curing. *J Contemp Dent Pract,* 13(5), 644-649.

Davidson, C.L., De Gee, A.J., Feilzer, A., 1984. The competition between the composite dentin bond strength and the polymerisation contraction stress. *J Dent Res*, 63(12), 1396-1399.

Davila, J.M., Gwinnett, A.J., Robles, J.C., 1986. Marginal adaptation of composite resins and dental bonding agents. *Journal of Dental Research* 65 *Abstracts of Papers* p 238 Abstract 623

Davila, J.M., Gwinnett, A.J., Robles, J.C., 1988. Marginal adaptation of composite resins and dental bonding agents. *Journal of Dentistry for Children,* 55, 25-28.

De Marsillac, M.D.W.S., Delbem, A.C.B., Vieira, R.D.S. 2008. Effect of time in hardness test on artificially demineralized human dental enamel. *Brazilian Journal of Oral Sciences,* 7, (24), 1507-1511.

Dickens, S.H., Flaim, G.M., 2008. Effect of a bonding agent on in vitro biochemical activities of remineralizing resin-based calcium phosphate cements*. Dental Materials,* 24 (9), 1273–1280.

Douglas, W.H., Chew, C.J., Craig, R.G., 1980. Neutron activation analysis of micro-leakage around a hydrophobic composite restorative. *Journal of Dental Research,* 59 1507-1510.

Ensaff, H., O'Doherty, D.M., Jacobsen, P.H., 2001. Polymerization shrinkage of dental composite resins. *Proc InstnMechEngrs, 215 (4), 367-375***.**

Ewoldsen, N., Demke, R.S., 2001. A review of orthodontic cements and adhesives. *American Journal of Orthodontics and Dentofacial Orthopedics*, 120, 45-48.

Fabianelli, A., Pollington, S., Davidson, C., Cagidiaco, M.C. and Goracci, C., 2007. The relevance of microleakage studies. *International Dentistry SA*, *9*(3), 64-74.

Fakiha, Z.A., Mueninghoff, L.A., Leinfelder, K.F., 1992. Rapid mixing of zinc phosphate cement for fixed prosthodontic procedures. *J Prosthet Dent,* 67, 52–58.

Featherstone, J.D.B., 1999. Prevention and reversal of dental caries: role of low level fluoride. *Community Dent Oral Epidemiol*, 27, 31-40.

Fejerskov, O., Manji, F., 1990. Reactor paper: Risk assessment in dental caries. In: Bader JD, ed. Risk assessment in dentistry. *Chapel Hill: University of North Carolina Dental Ecology,* 215-217*.*

Ferracane, J.L., Condon, J.R., Mitchem, J.C., 1992. Evaluation of subsurface defects created during the finishing of composites. *Journal of Dental Research,* 71, 1628-1632.

Ferracane, J.L., Mittchem, J.C., 2003. Relationship between composite contraction stress and leakage in class V cavities*. Am J Dent,* 16,239–243.

Ferracane, J.L., Stansbury, J.W., Burke, F.J.T., 2011. Self-adhesive resin cements–chemistry, properties and clinical considerations. *Journal of oral rehabilitation*, *38*(4), 295-314.

Forsten, L., 1998. Fluoride release and uptake by glass-ionomers and related materials and its clinical effect**.** *Biomaterials, 19,503–508*.

Fricker, J.P., 1997. A 12-month clinical comparison of resin-modified light activated adhesives for the cementation of orthodontic molar bands. *American Journal of Orthodontics and Dentofacial Orthopaedics,* 112**,** 239–243.

Fricker, J.P., 1989. A 12-month clinical study comparing four glassionomer cements for the cementation of orthodontic molar bands. *Aust Orthod J,* 11, 10-13.

Fuks, A.B., Holan, G., Simon, H., Lewinstein, I., 1992. Micro-leakage of class 2 glass ionomer- silver- restoration in primary molars, *Oper Dent,* 17, 62-69.

Galan J, J.r., Mondelli, J., Coradazzi, J.L., 1976. Marginal leakage of two composite restorative systems. *Journal of Dental Research,* 55, 74-76.

Gale, M.S., Darvell, B.W., 1999. Dentin permeability and tracer tests. *Journal of Dentistry,* 27(1), 1-11.

Georges, S.t.A.J., Wilder, J.r.D.A., Perdigao, J., Swift, J.r.J.E., 2002. Micro-leakage of class V composites using different placement and curing techniques: an in *vitro study*. *American Journal of Dentistry,* 15, 244–247.

Gillgrass, T., Millett, D.T., Creanor, S.L., Mackenzie, D., Bagg, J., Gilmour, W.H., 1999. Fluoride release, microbial inhibition and micro-leakage pattern of two orthodontic band cements. *J Dent,* 27, 455-461.

Goje, S.K., Sangolgi, V.K., Neela, P., Lalita, C.H., 2012. A comparison of resistance to enamel demineralization after banding with four orthodontic cements: an in vitro Study. *J Indian Ortho Soc,* 46,141–147.

Going, R.E., 1972. Micro leakage around dental restorations: a summarizing review *Journal of the American Dental Association,* 84, 1349-1357.

Going, R.E., Massler, M., Dute, H.L., 1960. Marginal penetration of dental restorations by different radioactive isotopes. *Journal of Dental Research,* 39, 273-284.

Going, R.E., Myers, H.M., Prussin, S.G., 1968. Quantitative method for studying micro-leakage in vivo and in vitro. *Journal of Dental Research* 47, 1128-1132.

Gorelick, L., Geiger, A.M., Gwinnett, A.J 1982. Incidence of white spot formation after bonding and banding.*Am J Orthod,* 81, 93–98.

Grobler, S.R., Rossouw, R.J., Kotze, T.J.V., 1998. A comparison of fluoride release from various dental materials. *J Dent, 26, 259–265.*

.Grobler, S.R., Rossouw, J., Oberholzer, G., 2007. Micro-leakage and confocal laser studies of 2 single-step self-etching bonding agents/ systems. *Quintessence Int*, 38,528,334-341.

Gwinnett, A.F., Ceen, R.F., 1979. Plaque distribution on bonded brackets: a scanning microscope study. *Am J Orthod, 75,667*-677.

Hadler-Olsen, S., Sandvik, K., El-Agroudi, M.A., Ogaard, B., 2012. The incidence of caries and white spot lesions in orthodontically treated adolescents with a comprehensive caries prophylactic regimen—a prospective study**.** *J* Eur *Orthod*, 34 (5)**,** 633-639.

Hallgren, A., Oliveby, A., Twetman, S., 1992. Caries associated in microflora in plaque from orthodontic appliances retained with glass ionomer cement. *Scand J Dent Res*, 100, 140-143.

Hammesfahr, P.D., Huang, C.T., Shaffer, S.E., 1987. Micro-leakage and bond strength of resin restorations with various bonding agents. *Dental Materials,* 3, 194-199.

Hanks C.T., Craig, R.G., Diehl, M.L., Pashley, D.H. 1988. Cytotoxicity of dental composites and other materials in a new in vitro device. *Journal of Oral Pathology,* 17, 396-403.

Harper, W.E., 1912. The character of the adaptation amalgam to the walls of cavities attained by present methods of instrumentation and the use of the best known alloys, as indicated by the air pressure test. *Dental Review,* 26, 1179-1198.

Hembree, J.H., Andrews, J.T., 1978. Micro-leakage of several class V anterior restorative materials: a laboratory study. *Journal of the American Dental Association,* 97, 179- 183.

Herrera, M., Castillo, A., Bravo, M., Lièbana, J., Carrion, P., 2000. Antibacterial activity of resin adhesive, glass ionomer and resin-modified glass ionomer cements and a compomer in contact with dentin caries samples. *Op Dent,* 25, 265-269.

Higham, S., 2014. Caries Process and Prevention Strategies: Demineralization/Remineralization. *Crest® + Oral-B® at dentalcare.com Continuing Education Course,*

Hilton, T.J., 2002. Can modern restorative procedures and materials reliably seal Cavities? *In vitro* investigations Part 1. *American Journal of Dentistry,* 15(3), 198-210.

Hodge, H.C., 1936. Hardness tests on teeth. *J Dent Res,* 15, 271-279.

Hu, W., Featherstone, J.D., 2005. Prevention of enamel demineralization: an *in-vitro* study using light cured filled sealant. *Am J Orthod Dentofacia Orthop, 128,592–600*.

James, J.W., Miller, B.H., English, J.D., Tadlock, L.P., Buschang, P.H., 2003. Effects of high speed curing devices on shear bond strength and micro-leakage of orthodontic brackets. *American Journal of Orthodontics and Dentofacial Orthopedics, 123,555–561.*

Joseph, V.P., Rossouw, E., 1990. The shear bond strengths of stainless steel and ceramic brackets used with chemically and light-activated composite resins. *Am J OrthodDentofacialOrthod,* 97, 121–125.

Jou, G.E., Leung, R.L., White, S.N., Zernik, J.H., 1995. Bonding ceramic brackets with light-cured glass ionomer Cements. *J ClinOrthod,* 3, 184–187.

Kakar, R.C., Subramanian, V., 1963. Sealing qualities of various restorative materials.*J Prosthet Dent,* 13,156-65.

Klock, B., Krasse, B., 1979. A comparison between different methods for prediction of caries activity. *Scand J Dent Res,* 1(87), 129-139.

Kidd, E.A., 1976. Micro-leakage: a review. *J Dent,* 4, 199-206

Knoop, P., Peters, G.C., Emerson, W.B., 1939. A sensitive pyramidal tool for indentation measurements. *J Res Nat Bur Stand,* 23, 39-61.

Kornfield, B., 1953. Study of clinical behaviour of resins in operative dentistry. *Journal of Dental Research* 32 *Abstracts of Papers* p 714 Abstract 208.

Loher, H., Behr, M., Hintereder, U., Rosentritt, M., Handel, G., 2009. The impact of cement mixing and storage errors on the risk of failure of glass – ceramic crowns. Clin. *Oral Investig*, 13, 217.

Loguercio, A.D., Roberto de Oliveira Bauer, J., Reis, A., Miranda Grande, R.H., 2004.In vitro micro-leakage of packable composites in Class II restorations. *Quint Int,* 35(1), 29-34.

Londono, J., Abreu, A., Nelson, S., Hernandez, J., Torres, C., Mettenburgf, D., Looney, S., Rueggeberg, F., 2009. Effect of vitaltooth bleaching on solubility and roughness of dental cements. *J Prosthet. Dent*, 102, 148.

Lundstrom, F., Krasse, B., 1987. *Streptococcus mutans* and *lactobacilli* frequency in orthodontic patients; the effect of chlorhexidine treatments. *Eur J Orthod*, 9, 109-116.

Majeed, A., 2005*. AN IN Vitro study of micro-leakage and surface micro-hardness of nanocomposite restorative materials.* Thesis (MSc). University of Western Cape.

Mali, P., Deshpande, S., Singh, A., 2006. Micro-leakage of restorative materials: an *in vitro* study. *Journal of the Indian Society of Pedodontics and Preventive Dentistry,* 24, 15 – 18.

Maltz, M., Emilson, C.G., 1982. Susceptibility of Oral Bacteria to Various. *J Dent Res,* 61(6), 786-790.

Marcusson, A., Norevall, L.I., Persson, M., 1997. White spot reduction when using glass ionomer cement for bonding in Orthodontics: a longitudinal and comparative study. *Eur J Orthod*, 19, 233-242.

Martignon, S., Ekstrand, K.R., Lemos, M.I., Lozano, M.P., Higuera, C., 2010. Plaque caries level and oral hygiene habits in young patients receiving orthodontic treatment. *Community Dent Health*, 27,133–8.

Mason, D., Kuo, E., 2010. Dental appliance with simulated teeth and method for making Align Technology Inc. Patent Application Puplication.

Matalon, S., Slutzky, H., Weiss, E.I., 2005. Antibacterial properties of four orthodontic cements. *Am J Orthod Dentofac Orthop,* 127, 56-63.

Mathew, M., Paramaswaran Nair, E.K., Krishnan, K., 2001. Bonding agent is a decisive factor in determining the marginal leakage of dental Composites subjected to thermal cycling: an in vitro study*, J Oral Rehab,* 28, 68-77.

McCabe, J.F., Walls, A.W., 1998. Properties used to characterize materials. Applied Dental Materials. *8th ed Oxford: Blackwell Science*, 4-28.

McCourt, J.W., Cooley, R.L., Barnwell, S., 1991. Bond strength of light-cure fluoride-releasing base-liners as orthodontic bracket adhesives. *Am J Orthod Dentofacial Orthop* 100, 47-52.

McLean, J.W., Wilson, A.D., Prosser, H.J., 1984. Development after use of water hardening glass ionomer luting cements. *Journal of Prosthetic Dentistry*, 52, 175-181.

Menaker, L., 1980. The Biologic Basis of Caries, Harper and Row, Cambridge.

Mennemeyer, V.A., Neuman, P., and Powers, J.M., 1999. Bonding of hybrid ionomers and resin cements to modified orthodontic band materials*. American Journal of Orthodontics and Dentofacial Orthopaedics,* 115, 143–147.

Meyer, J.M., Dennison, J.B., Craig, R.G., 1974. Improved method of neutron activation analysis for micro-leakage studies. *Journal of Dental Research,* 53,356-363.

Moller, B., Schroder, U., Granath, L., 1983. Effect of IRM on human dental pulp. *Scandinavian Journal of Dental Research,* 91, 281-287.

Millett, D.T., McCabe, J.F., 1996. Orthodontic bonding with glass ionomer cement-areview. *European Journal o] Orthodontics,* 18, 385-399**.**

Millett, D.T., Cattanach, D., McFadzean, R., Pattison, J., McColl, J., 1999. Laboratory evaluation of a compomer and a resin-modified glass ionomer cement for orthodontic bonding. *Angle Orthod*, 69, 58-63.

Millett, D., Mandallb, N., Hickmanc, J., Mattickd, R., Glennye, A.M., 2009. Adhesives for Fixed Orthodontic Bands. *Angle Orthod,* 79,193–199.

Miller, J.R., Mancl, L., Arbuckle, G., Baldwin, J., Phillips, R.W., 1996. A three-year clinical trial using a glass ionomer cement for the bonding of orthodontic brackets. *Angle Orthod*, 66,309-312.

Miyazaki, M., Hinoura, K., Onose, H., Moore, B.K., 1991.Effect of filler content of light-cured composites on bond strength to bovine dentine. *J Dent,* 19,301–303.

Moosavi, H., Ahrari, F., Mohamadipour, H., 2013.The effect of different surface treatments of demineralised enamel on micro-leakage under metal orthodontic brackets*. Progress in Orthodontics,* 14(1), 2, 14-2

Mortensen, D.W, Boucher, N.E., Ryge, G., 1965. A method of testing for marginal leakage of dental restorations with bacteria .*Journal of Dental Research,* 44, 58-63.

Mulder, R., Grobler, S.R., and Osman, Y.I., 2013. Volumetric change of flow-able composite resins due to polymerization as measured with an electronic mercury dilatometer. *Oral Biology and Dentistry*, 1, 1.

Musanje, L., Darvell, B.W., 2004. Effects of strain rate and temperature on the mechanical properties of the resin composites. *Den,* 20 (8), 750–765

Namboori, R., Gandikota, C.S., Challa, P.L., Juvvadi, S.R., Palla, Y., Gutti, H.R., 2012. Comparison of micro-leakage in metal brackets bonded using self-etching adhesive with and without fluoride release: An in *vitro study. J IndOrthoodSoc*, 46 (4), 245- 249.

Newbrun, E., 1989. Cariology. 3rd edn*. Chicago: Quintessence*, 29-61.

Neira, I.S., Kolen'ko, Y.V., Lebedev, O.I., Van Tendeloo, G., Gupta, H.S., Matsushita, N., Yoshimura, M., Guitia´ n, F., 2009. Rational synthesis of a nanocrystalline calcium phosphate cemen exhibiting rapid conversion to hydroxyapatite. *Mater. Sci. Engin,C* 29, 2124.

Newman, G.V., 1965. Epoxy adhesive for orthodontic attachments. Progress report. *American Journal of Orthodontics,* 51, 901-912.

Nkosi, P.M., 2008. *Efficacy of enamel sealants in prevention of demineralization.* Thesis (MSc).University of Western Cape.

Nicholson, J.W., McKenzie, M.A., Goodrige, R., Wilson, A.D., 2001. Variations in the compressive strength of dental cements stored in ionic or acidic solutions. *J. Mater. Sci. Mater. Med.* 12, 647.

O'Brien, W.J., 2002. Dental Materials and Their Selection, 3rd ed. *Quintessence Publishing Co. Inc,* 136*.*

O'Brien, W.J., 2002. Dental material and their selection. *Quintessence publishing Co, Inc,* (3), 17-18.

Oberholzer, T.G., Du Preez, I.C., kidd, M., 2005. Effect of LED curing on the micro-leakage, shear bond strength and surface hardness of a resin-based composite restoration. *Biomaterials,* 26(18), 3981–3986.

.Oen, O., Gjerdet, N.R., Wisth, P.J, 1991. Glass ionomer cements used as bonding materials for metal orthodontic brackets an *in-vitro* study. *European Journal of Orthodontics,* 13, 187-191.

Øgaard, B., 1989. Prevalence of white spot lesions in 19-year-olds: a study on untreated and orthodontically treated persons 5 years after treatment. *Am J OrthodDentofacOrthop,* 96, 423-427.

Øgaard, B.E., Larsson, T., Bishara, H.S., 2001. Effects of combined application of antimicrobial and fluoride varnishes in orthodontic patients. *Am J Orthod Dentofacial Orthop*, 120, 28–35

Olivia, A., Della Ragione, F., Salerno, A., 2000. Biocompatibility studies on glass ionomer cements by primary cultures of human osteoblasts. *Biomaterials,* 17, 1351–1356.

Osorio, R., Toledano, M., Garcı´a-Godoy, F., 1998. Enamel surface morphology after bracket debonding. *J Dent Child,* 65(5), 313–317.

Paschos, E., Kleinschrodt, T., Clementino- Luedemann, T., Huth, K.C., Hickel, R., Kunzelmann, K.H., Rudzki-Jansong, I., 2009. Effect of different bonding agents on prevention of enamel demineralization around orthodontic brackets. *Am J Orthod Dentofacial Orthop,* 135,603-612.

Patil, P., Kaur, S., Kaur, M., Kaur, M., Vinuta, S., Kaur, R.K., 2014. Orthodontic Cements and Adhesives: A Review*. Journal of Advanced Medical and Dental Sciences Research, 2, 3.*

Phair, C.B., Fuller, J.L., 1985. Micro-leakage of composite resin restorations with cementum margins. *J Prosthet Dent,* 53,361-364*.*

Poskus, L.T., Placido, E., Cardoso, P.E.C., 2004. Influence of placement techniques on Vickers and Knoop hardness of class II composite resin restorations*. Dent Mater,* 20(8), 726-732.

Powis, D.R., Prosser, H.J., Shortall AC, Wilson, A.D., 1988. Long-term monitoring of micro-leakage of composites. Part I: Radiochemical diffusion technique. *Journal of Prosthetic Dentistry,* 60, 304-307.

Puckett, A.D., Fitchie, J.G., Bennett, B., Hembree, J.H., 1995. Micro-leakage and thermal properties of hybrid ionomer restoratives. *Quintessence International,* 26, 583-585.

Purdell-Lewis D.J., Groeneveld, A., Arends, J., 1976. Micro-hardness and densitometric measurements of the effect of 4% SnF2 solution on artificial white spot lesions*. Caries Res, 10, 216-26.*

Pradeep, S., Shanthraj Ravi Jyothi, H., Kiran Shivalinga, B.M., 2013. Comparative Evaluation of the Shear Bond Strength and De-bonding Properties of a Conventional Composite and Flowable Composites used for Orthodontic Bracket Bonding. *World Journal of Dentistry*, 4(1), 6-16.

Prati, C., Nucci, C., Montanari, G., 1991. Shear bond strength and micro-leakage of dentin bonding systems. *J Prosthet Dent,* 65, 401-407.

Prosser, H.J., Powis, D.R., Brant, P., Wilson, A.D., 1984. Characterization of glass-ionomer cements the physical properties of current materials. *J Dent,* 12,231-40.

Ramoglu, SI.., Uysal, T., Ulker, M., Ertas, H., 2009. Micro-leakage under ceramic and metallic brackets bonded with resin-modified glass ionomer. *Angle Orthodontist,* 79, 138–143.

Retief, D.H., Dreyer, C.J., Garvon, G., 1970. The direct bonding of orthodontic attachments to teeth by means of an epoxy resin adhesive. *American Journal of Orthodontics,* 58, 21-40.

Retief, D.H., 1994. Do adhesives prevent micro-leakage? *International Dental Journal,* 44(1), 19-26.

Rix, D., Foley, T.F., Mamandras, A., *2001***.** *Comparison of bond strength of three adhesives: Composite resin, hybrid GIC, and* glass-filled GIC*. Am J Orthod Dentofacial Orthop,* 119, 36-42

Rossouw, P.E., Penuvchev, A.V., Kulkarni, K., 1996. The influence of various contaminants on the bonding of orthodontic attachments. *Ont Dent*, 73, 15-22.

Rockwell Hardness Testing, 2010. [*http://hardnesstesters.com/Applications/Rockwell-Hardness-Testing.aspx*](http://hardnesstesters.com/Applications/Rockwell-Hardness-Testing.aspx)*. Retrieved on 11th July, 2016*

Rossouw, P.E., 2010. A historical overview of the development of the acid-etch bonding system in orthodontics. *In Seminars in orthodontics*, 16(1) 2-23.

Rueggeberg, F.A. 1991*.* Substrate for adhesion testing to tooth structure-review of the literature*. Dental Materials,* 7, 2-10.

Sabzevari, B., Ramazanzadeh, B.A., Moazzami, S.M., 2013. Micro-leakage under Orthodontic Metal Brackets Bonded with Three Different Bonding Techniques with/without Thermo-cycling*. J Dent Mater Tech,* 2(1), 21-28.

Schmalz, G., 2009. Resin-Based Composites. *Biocompatibility of Dental Materials,* 99-113.

Shortall, A.C., 1982. Micro-leakage marginal adaptation and composite resin restorations. *Br Dent J,* 153, 223-227*.*

Sideridou, I., Achilias, D., Kyrikou, E., 2004. Thermal expansion characteristics of light-cured dental resins and resin composites. *Biomaterials,* 25, 3087 – 3097

Sidhu, S.K., Carrick, T.E., McCabe, J.F., 2004. Temperature mediated coefficient of dimensional change of dental tooth-colored restorative materials. *Dental Materials,* 20, 435-440.

Silverman, E., Cohen, M., Demke, R.S., Silverman, M., 1995. A new light-cured glass ionomer cement that bonds brackets to teeth without etching in the presence of saliva. *American Journal of Orthodontics and Dentofacial Orthopedics,* 108, 231–236

Siqueira, J.F.J.r., Rocas, I.N., Abad, E.C., Castro, A.J., Gahyva, S.M., Favieri, A., 2001. Ability of three root-end filling materials to prevent bacterial leakage. *J Endod*, 27(11), 673-675.

Smith, R.L., Sandland, G.E., 1922. "An Accurate Method of Determining the Hardness of Metals, with Particular Reference to Those of a High Degree of Hardness," *Proceedings of the Institution of Mechanical Engineers* I, 623–641.

Smales, R.J., 1981. Plaque growth on dental restorative materials. *J Dent, 9,133-40*.

Sudjalim, T.R., Woods, M.G., Manton, D.J., 2006. Prevention of white spot lesions in orthodontic practice: a contemporary review. *Aust Dent J*, 51, 284-289.

Summitt, J.B., Robbins, J.W, Schwartz, R.S., 2006. Fundamentals of Operative Dentistry: A Contemporary Approach. *3 rd ed., Ch. 1. Hanover Park, IL: Quintessence Publishing,* 2-4.

Summitt, J.B., Robbins, J.W., Schwartz, R.S., 2006. Fundamentals of Operative Dentistry: A Contemporary Approach, 3rd ed. Hanover Park, IL, *Quintessence Publishing,* 2-4.

Staninec, M., Mochizuki, A., Tanizaki, K., Jukuda, K., Tsuchitani, Y., 1986. Interfacial space, marginal leakage and enamel cracks around composite resins. *Op Dent*; 11, 14-24.

Tabor, D., 2000. *The hardness of metals*. Oxford university press.

Tamizharasi, Kumar, S., 2010.Evolution of Orthodontic Brackets. *JIADS,* 1 (3), *25-30.*

Tate, W.H., You, C., Powers, J.M., 2000. Bond strength of compomers to human enamel. *Oper Dent*, 25,283-91.

Taylor, M.J., Lynch, E.L., 1992. Micro-leakage. *Journal of Dentistry,* 20, 3-10.

Thonemann, B.M., Federlin, M., Schmalz, G., 1997. SEM analysis of marginal expansion and gap formation in Class II composite restorations. *Dent Mater*, 13, 192-197.

Tavas, M.A., Watts, D.C., 1979. Bonding of orthodontic brackets by transillumination of a light activated composite: an in vitro study. *Br J Orthod,* 207–208.

Taylor, M.J., Lynch, E. 1992. Micro-leakage. *Journal of Dentistry,* 20 (1), 3-10.

Tecco, S., Traini, T., Caputis, Festa, F., de Luca, V., D’Attilio, M., 2005. A new one-step dental flowable composite for orthodontic use: an *in vitro* bond strength study. *The Angle Orthodontist,* 75, 672 – 677.

Tjan, A., Tan, D., 1991. Micro-leakage at gingival margins of class V composite resin restorations rebounded with various low-viscosity resin systems. *Quintessence International,* 22 (7), 565-573.

Toledano, M., Osorio, R., Osorio, E., Romeo, A., Higuera, B.d.l., Garcı´a-Godoy, F., 2003. Bond Strength of Orthodontic Brackets Using Different Light and Self-Curing Cements. *Angle Orthod*, 73, 56–63.

Torii, Y., Itota, T., Okamoto, M., Nakabo, S., Nagamine, M., and Inone, K., 2001. Inhibition of artificial secondary caries in root by fluoride-releasing restorative materials. *Oper Dent,* 26-36.

Trimpeneers, L.M, Verbeeck, R.M.H., Dermaut. L.R., Moors. M.G., 1996. Comparative shear bond strength of some orthodontic bonding resins to enamel. *Eur J Orthod,* 18, 89–95.

Ulker, M., Uysal, T., Ramoglu, S.I., Ertas, H., 2009. Micro-leakage under orthodontic brackets using high-intensity curing lights. *Angle Orthod*, 79,144-149.

Uysal, T., Ulker, M., Ramoglu, S.I., Ertas, H., 2008. Micro-leakage undermetallic and ceramic backets bonded with orthodontic self-etching primer systems. *Angle Orthod,* 6,1089–1094.

Uysal,T., Ramoglu, S.I., Ertas, H., Ulker, M., 2010. Micro-leakage of orthodontic band cement at the cement-enamel and cement-band interfaces. *American Journal of Orthodontics and Dentofacial Orthopedics,* 137, 4534–4539.

Van Dijken, J.W.V., Hors, Ted. B.P., 1989. The effects of Gluma and Gluma/Scotchbond on in vivo marginal adaptation of a composite resin to dentin. *Dental Materials,* 5, 165-167

Van Houte, J., 1980. Bacterial specificity in the etiology of dental caries. *Int Dent J*, 30,305-326.

Van Landuyt, K.L., Yoshida, Y., Hirata, I., Snauwaert, J., De Munck, J., Okazaki, M., *et al.,* 2008. Influence of the chemical structure of functional monomers on their adhesive performance. *J Dent Res*, 87,757–761.

Vicente, A., Ortiz, A.J., Bravo, L.A., 2009. Micro-leakage beneath brackets bonded with fltowable materials: effect of thermos-cycling. *European Journal of Orthodontics* 31, 390–396

Vicente, A., Navarro, R., Mena, A., Bravo, L., 2006. Effect of surface treatments on the bond strength of brackets bonded with a compomer. *American Journal of Dentistry,* 19, 271– 274.

Vickers Hardness Testing, 2010*.* *http://hardnesstesters.com/Applications/Vickers-Hardness-Testing.aspx*. *Retrieved on 11th July, 2016*

Vorhies, A.B., Donly, K.J., Staley, R.N., Wefel, J.S., 1998. Enamel demineralisation adjacent to orthodontic brackets bonded with Hybrid glass ionomer cements: an *in-vitro* study*. Am J OrthodDentofacOrthop,* 114, 668-674.

Wagh, Arun, S., 2004. Chemically Bonded phosphateceramics. *Twenty- First Century Materials with Diverse Applications. Elsevier.*

Wahab, F.K., Shaini, F.J., Morgano, S.M., 2003. The effect of thermos-cycling on micro-leakage of several commercially available composite class V restoration *in vitro*. *Journal of Prosthetic Dentistry,* 90 (2), 168-174.

Weerheijm, K.L., 2003. Molar incisor hypomineralisation (MIH). *Eur J Paediatr Dent,* 4,114–120.

White, D.J., Faller, R.V., Bowman, W.D., 1992. Demineralisation and remineralisation evaluation techniques. Added considerations. J Dent Res, 71, 929-933.

Wibowo, G., Stockton, L., 2001. Micro-leakage of class II composite restorations. *Am. J. Den,* 14 (3), 177-185.

Wilson, A.D., Kent, B.E., *1972*. A new translucent cement for dentistry the glass ionomer cement *Br Dent J,*132, 133–5.

Wilson, R.M., Donly, K.J**.,** 2001. Demineralization around orthodontic brackets bonded with resin-modified glass ionomer cement and fluoride-releasing resin composite. *Pediatr Dent,* 23, 255-259, Yagci, A., Uysal, T.A., Ulker, M., Ramoglu, S.I., 2010. Micro-leakage under orthodontic brackets bonded with the custom base indirect bonding technique. *European Journal of Orthodontics,* 32, 259–263.

Yavuz, I., Aydin, H., 2005. New method for measurement of surface areas of micro-leakage at the primary teeth by biomolecule characteristics of methylene blue. *Biotechnology and Biotechnological Equipment,* 19 (1), 181-187.

Yazici, A.R., Baçeren, M., Dayangaç, B., 1985. Oral fluid contamination of etched enamel surfaces: An SEM study. *J Am Dent Assoc,* 110, 329-32.

Yazici, A.R., Baçeren, M., Dayangaç, B., 2002. The effect of current-generation bonding systems on micro-leakage of resin com-positetestorations. *Quintessence Int,* 33,763-9.

Youngson, C.C., Grey, N.J., Martin, D.M., 1990. In vitro marginal micro-leakage associated with five dentine bonding system and associated composite restorations. *J Dent,* 18, 203-8.

Youngson, C.C., 1992. A technique for three-dimensional Micro-leakage assessment using tooth sections. *J. Dent.*, 20(4), 231-234.

Zimmer, W.B., Rollwinkel, Y., 2004. Assessing patient-specific decalcification risk in fixed orthodontic treatment and its impact on prophylactic procedures. *Am J OrthodDentofacialOrthop,* 126, 318–24.

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Chapter 10

Appendix

Appendix

1. Consent

Dear Patient,

Dr Marrow Elshami is doing research on “dental materials used for the treatment of tooth”. In order to conduct this research, extracted premolar teeth are required. These teeth will be incinerated after the research has been conducted. Patient confidentiality will be preserved. By signing this from you grant permission for your teeth to be used for research purposes.

Signature…………………. Date……….

2. Micro-leakage:

Statistics result as recommended by statistician.

	group 1 Fuji Ortho LC		1mm=40mm xx	
samples	occlusal bracket-adhesive	gingival bracket-adhesive	occlusal enamel-adhesive	gingival enamel-adhesive
1	21.67	35.16	28.69	54.05
2	17.30	126.0	18.93	126.0
3	0	6.75	0	7.04
4	24.61	118.79	88.05	118.97
5	48.73	131.84	48.99	131.84
6	59. 64	71.20	59.64	71.20
7	9.73	22.60	9.73	22.60
8	33.64	61.68	63.85	93.62
9	137.03	138.20	43.26	138.20
10	79.31	79.84	127.86	139.16
11	31.82	51.01	32.59	51.01
12	7.02	16.56	11.94	16.56
13	30.99	60.20	47.99	69.20
14	30.72	59.76	30.72	59.76
15	33.27	41.42	33.27	41.42
16	137.50	141.15	137.50	141.15
17	135.32	136.0	135.32	136.0

18	35.04	54.90	25.04	27.91
19	87.19	99.15	30.95	80.99
20	70.51	107.30	70.51	107.30

group 1 Fuji Ortho LC				
samples	occlusal bracket-adhesive	gingival bracket-adhesive	occlusal enamel-adhesive	gingival enamel-adhesive
1	0.5	0.8	0.7	1.3
2	0.4	3.15	0.4	3.15
3	0	0.1	0	0.1
4	0.6	2.9	2.2	2.9
5	1.2	3.2	1.2	3.2
6	1.4	1.7	1.4	1.7
7	0.2	0.5	0.2	0.5
8	0.8	1.5	1.5	2.3
9	3.4	3.4	1.0	3.4
10	1.9	1.9	3.1	3.4
11	0.7	1.2	0.8	1.2
12	0.1	0.4	0.2	0.4
13	0.7	1.5	1.1	1.7
14	0.7	1.4	0.7	1.4
15	0.8	1.0	0.8	1.0
16	3.4	3.5	3.4	3.5

17	3.3	3.4	3.3	3.4
18	0.8	1.3	0.6	0.6
19	2.1	2.4	0.7	2.0
20	1.7	2.6	1.7	2.6

	group 2 RelyX luting 2	1mm=40mm xx		
samples	occlusal bracket-adhesive	gingival bracket-adhesive	occlusal enamel-adhesive	gingival enamel-adhesive
1	88.10	127.79	88.10	127.79
2	58.45	87.80	58.45	86.88
3	75.30	120.26	95.22	120.26
4	53.80	62.93	53.80	62.93
5	126.09	132.50	100.09	99.0
6	118.57	137.68	118.57	137.68
7	130.88	132.76	130.88	132.76
8	48.17	114.71	49.43	58.17
9	74.56	126.23	58.25	126.23
10	99.02	131.75	124.61	131.75
11	135.10	137.47	135.10	137.47
12	127.30	133.56	127.30	130.67
13	132.10	136.60	52.01	136.60
14	135.0	135.76	114.11	115.11

15	75.12	79.01	75.12	79.01
16	126.93	130.57	126.93	144.57
17	23.61	96.86	65.25	114.86
18	71.58	73.38	51.48	106.61
19	77.91	78.48	77.91	78.48
20	110.74	113.65	130.81	136.64

	group 2 Rely X luting 2			
samples	occlusal bracket-adhesive	gingival bracket-adhesive	occlusal enamel-adhesive	gingival enamel-adhesive
1	2.2	3.1	2.2	3.1
2	1.4	2.1	1.4	2.1
3	1.8	3.0	2.3	3.0
4	1.3	1.5	1.3	1.5
5	3.1	3.3	2.5	2.4
6	2.9	3.4	2.9	3.4
7	3.2	3.3	3.2	3.3
8	1.2	2.8	1.2	1.4
9	1.8	3.1	1.4	3.1
10	2.4	3.2	3.1	3.2
11	3.3	3.4	3.3	3.4
12	3.1	3.3	3.1	3.2

13	3.3	3.4	1.3	3.4
14	3.3	3.3	2.8	2.8
15	1.8	1.9	1.8	1.9
16	3.1	3.2	3.1	3.6
17	0.5	2.4	1.6	2.6
18	1.7	1.8	1.2	2.6
19	1.9	1.9	1.9	1.9
20	2.7	2.8	3.2	3.4

	group 3 Transbond XT		1mm=40mm xx	
samples	occlusal bracket-adhesive	gingival bracket-adhesive	occlusal enamel-adhesive	gingival enamel-adhesive
1	0	0	0	18.13
2	0	0	0	0
3	0	0	0	0
4	0	0	0	0
5	0	0	0	0
6	0	0	0	0
7	0	0	0	0
8	0	0	0	25.30
9	0	5.90	0	0
10	0	0	0	0
11	0	0	0	56.14

12	0	0	0	88.51
13	0	0	0	0
14	0	0	0	32.34
15	0	0	0	0
16	10.94	13.45	10.94	13.45
17	0	0	0	22.96
18	0	8.58	0	14.02
19	0	0	0	0
20	0	0	32.94	33.99

	group 3 transbond XT			
samples	occlusal bracket-adhesive	gingival bracket-adhesive	occlusal enamel-adhesive	gingival enamel-adhesive
1	0	0	0	0.4
2	0	0	0	0
3	0	0	0	0
4	0	0	0	0
5	0	0	0	0
6	0	0	0	0
7	0	0	0	0
8	0	0	0	0.6
9	0	0.1	0	0
10	0	0	0	0

11	0	0	0	1.4
12	0	0	0	2.2
13	0	0	0	0
14	0	0	0	0.8
15	0	0	0	0
16	0.2	0.3	0.2	0.3
17	0	0	0	0.5
18	0	0.2	0	0.3
19	0	0	0	0
20	0	0	0.8	0.8

3. Demineralization:

1 pilot	Y	VHV		2 before	Y	VHV
	48.6 , 35.3	312			38.5 , 38.5	375
	45.5 , 37.7	321			44.9 , 43.1	287
	57.0 , 31.6	283			44.3 , 41.4	304
	63.0 , 47,1	184			43.3 , 43.1	298
	43.8 , 51,7	245			43.1 , 43.1	299

	50.3 , 42.6	258			43.2 , 43.2	298
	40.0 , 38,8	358			43.6 , 43.5	294
	51.6 , 36.8	285			43.4 , 43.4	295
	54,0 , 57.1	181			43.4 , 43.4	295
	50.7 , 50.7	216			43.4 , 43.0	298

3 before	Y	VHV		4 before	Y	VHV
	41.9 ,41.9	317			43.0 51.3	251
	43.9 , 38.2	331			43.5 , 45.0	285
	38.2 , 38.2	381			45.4 , 45.4	270
	40.1 , 40.2	346			42.4 , 45.7	287
	41.9 , 40.9	325			41.0 , 41.6	326

	41.5 , 41.1	326			45.5 , 45.5	269
	41.1 , 40.6	334			43.9 , 49.1	257
	42.2 , 39.0	337			45.8 , 39.6	305
	41.3 , 40.4	334			44.9 , 44.4	280
	43.5 , 39.5	323			39.9 , 43.9	317

5 before	Y	VHV		6 before	Y	VHV
	41.0 , 44.3	307			42.9 , 42.9	302
	45.7 , 45.7	266			46.8 , 46.5	256
	47.9 , 42.4	274			46.8 , 46.8	255
	45.7 , 43.8	278			46.1 , 46.4	261
	48.3 , 46.9	246			46.4 ,46.4	258

	48.2 , 48.2	239			43.3 , 43.3	297
	47.1 , 47.1	251			50.4 , 50.4	219
	47.4 ,47.1	250			50.4 , 48.6	227
	47.6 , 45.5	257			53.6 , 48.9	212
	39.2 , 48.7	290			47.9 , 47.9	246

7 before	Y	VHV		8 before	Y	VHV
	44.6 , 44.6	280			54.8 , 54.8	185
	44.6 , 44.6	280			55.0 , 55.0	184
	44.6 , 55.5	223			55.0 , 55.0	184
	45.4 , 43.4	282			45.0 , 47.8	258
	43.4 , 43.4	295			40.4 , 48.8	280

	43.5 ,43.5	294			44.0 , 44.0	287
	43.5 , 43.5	294			44.0 , 44.9	282
	51.1 , 47.0	232			40.7 , 41.3	331
	43.5 , 43.5	294			46.2 , 46.2	261
	39.3 , 45.5	314			46.2 , 46.2	261

9 before	Y	VHV		10 before	Y	VHV
	44.8 , 44.8	277			55.8 , 46.9	211
	44.8 , 43.2	287			56.2 , 39.5	243
	44.9 , 44.9	276			44.9 , 44.9	276
	44.6 , 44.9	278			50.2 , 46.5	238
	44.9 , 43.6	285			46.5 , 46.1	260

	42.1 , 42.1	314			52.4 , 48.7	218
	38.7 , 38.7	371			48.7 , 48.7	235
	42.1 , 42.1	314			48.7 , 48.7	235
	41.0 , 37.9	358			48.7 ,48.7	235
	42.3 , 39.6	333			48.7 , 48.7	235

11 before	Y	VHV		12 before	Y	VHV
	40.9 , 37.0	368			47.8 , 53.6	216
	48.7 , 40.4	281			39.6 , 39.6	355
	47.4 , 42.3	277			45.3 , 48.2	255
	44.3 , 42.6	295			43.7 , 43.7	291
	55.4 , 42.1	235			43.7 , 45.4	281

	43.1 , 41.3	312			49.9 , 49.4	226
	56.3 , 47.3	207			43.7 , 43.7	291
	59.2 , 39.3	230			41.8 , 47.9	277
	48.4 , 41.1	278			43.7 , 43.7	291
	52.5 , 42.5	247			45.3 , 45.3	271

13 before	Y	VHV		14 before	Y	VHV
	41.6 , 46.9	285			46.8 , 43.8	271
	39.1 , 45.3	312			43.0 , 43.0	301
	39.4 , 48.7	287			43.5 , 41.5	308
	36.4 , 40.8	373			41.5 , 41.5	323
	40.8 , 47.2	287			41.5 , 41.5	323

	46.6 , 46.6	256			41.5 , 41.5	323
	42.0 , 48.6	271			41.5 , 41.5	323
	40.3 , 47.4	290			41.5 , 41.5	323
	44.6 , 46.6	268			44.9 , 44.9	276
	45.5 , 49.0	250			44.9 , 44.9	276

15 before	Y	VHV		16 before	Y	VHV
	56.5 , 46.7	209			42.0 ,42.0	315
	56.5 ,46.7	209			42.1 , 42.1	314
	59.7 , 37.6	236			42.1 , 42.1	314
	49.3 ,37.5	295			42.1 , 42.1	314
	43.0 , 43.O	301			42.1 , 42.1	314

	52.0 , 44.2	262			45.3 , 42.0	293
	45.3 , 41.4	297			45.2 , 45.2	272
	45.4 , 42.0	291			44.1 , 44.1	286
	49.9 , 45.3	246			45.9 , 44.8	271
	52.5 , 40.6	257			44.8 , 42.6	291

17 before	Y	VHV		18 before	Y	VHV
	43.0 , 43.0	301			41.4 , 43.8	307
	42.8 , 42.1	309			44.3 ,44.3	283
	40.4 , 40.4	341			44.3 , 45.3	277
	42.7 , 42.7	305			44.9 , 44.9	276
	42.7 , 42.7	305			45.2 , 45.4	271

	42.7 , 38.6	337			45.4 , 42.5	289
	41.9 , 41.9	317			44.4 , 44.2	283
	41.9 , 41.9	317			45.9 , 43.8	277
	41.9 , 41.9	317			40.6 , 40.6	337
	41.4 , 41.4	325			40.6 , 40.6	337

19 before	Y	VHV		20 before	Y	VHV
	43.4 , 41.3	311			38.5 , 40.1	360
	45.9 , 43.2	281			44.7 , 44.7	278
	46.8 , 43.2	271			45.8 , 45.8	265
	45.8 , 42.7	285			47.5 , 43.9	266
	46.9 , 44.O	270			44.5 , 47.1	265

	46.0 , 39.8	302			43.9 , 46.0	276
	43.4 , 42.4	302			46.0 , 40.7	297
	43.2 , 40.1	321			42.8 , 38.4	337
	41.4 , 41.4	325			58.9 , 58.9	160
	43.1 , 41.8	309			43.1 , 43.0	301

21 before	Y	VHV		22 before	Y	VHV
	43.4 , 43.9	293			45. 5 , 43.1	283
	42.1 , 42.4	312			43.1 , 47.4	272
	42.5 , 42.5	308			45.7 ,47.4	257
	43.9 , 41.5	305			44.6 , 44.6	280
	45.0 , 43.8	282			43.3 , 46.6	276

	44.5 , 36.3	341			48.4 , 48.4	237
	45.1 , 42.4	291			41.0 , 41.3	329
	44.4 , 44.5	282			41.3 , 46.3	290
	42.1 , 42.1	314			46.3 , 44.3	271
	38.2 , 42.9	339			44.3 , 44.3	283

1 AFTER	Y	VHV		2 AFTER	Y	VHV
	43.3 , 44.0	293		Fuji Ortho LC	39.1 , 42.1	337
	45.4 , 45,4	270			42.1 , 42.1	314
	36.0 , 38.2	404			40.7 , 40.7	336
	37.2 , 37.2	402			39.2 , 39.2	362
	42.6 , 42.6	307			38.2 , 38.2	381

	42.6 , 42.6	307			43.5 , 43.5	294
	43.4 ,43.4	295			38.2 , 38.2	381
	41.8 , 40.4	329			40.3 , 40.3	343
	33.7 , 36.3	454			45.7 , 37.2	325
	42.0 , 36.4	362			41.5 , 41.5	323

3 AFTER	Y	VHV		4 AFTER	Y	VHV
Fuji Ortho LC	41.5 , 41.5	323		Fuji Ortho LC	40.6 ,40.6	337
	39.4 , 39.4	358			37.6 , 37.6	393
	39.0 , 40.6	351			41.3 , 41.3	326
	40.9 , 40.9	333			41.8 , 41.8	318
	40.4 , 40,4	341			45.8 , 45.8	365

	38.4 , 38.4	377			41.0 ,41.O	331
	35.9 , 36.8	422			45.7 , 44.2	276
	36.8 , 41.0	368			43.6 , 43.6	293
	40.0 , 40.0	348			43.6 , 43.6	293
	40.0 , 40.0	348			37.8 35,6	413

5 AFTER	Y	VHV		6 AFTER	Y	VHV
Fuji Ortho LC	44.3 , 46.5	270		Fuji Ortho LC	43.9 , 42.8	297
	45.9 , 39.3	307			42.8 ,42.8	304
	43.2 , 38.2	336			39.4 , 39.4	358
	39.2 , 37.2	381			38.2 , 38.2	381
	45.3 , 40.0	307			37.0 , 37.0	406

	45.7 , 43.0	283			39.5 39.5	357
	45.8 , 39.7	305			39.5 , 39.5	357
	47.4 , 45.0	261			39.5 , 39.5	357
	44.8 , 44.8	277			39.5 , 39.5	357
	44.8 , 44.8	277			39.5 , 42.4	333

7 after	Y	VHV		8 AFTER	Y	VHV
Fuji Ortho LC	39.5 , 39.5	357		Fuji Ortho LC	41.1 , 41.1	329
	40.1 , 40.1	346			42.0 , 42.0	315
	40.1 , 40.1	346			39.4 , 39.4	358
	40.1 , 40.1	346			42.9 , 42.9	302
	40.1 , 40.1	346			41.1 , 41.1	329

	40.1 , 40.1	346			42.3 , 40.4	326
	40.1 , 40,1	346			40.4 ,40.4	341
	38.2 , 38.2	381			39.5 , 39.5	357
	38.2 , 38.2	381			41.9 , 43.3	307
	38.2 , 41.7	349			43.3 , 42.4	304

9 AFTER	Y	VHV		10 AFTER	Y	VHV
Rely X luting 2	40.4 , 40.4	341		Rely X luting 2	41.6 , 37.0	360
	42.9 , 42.1	308			40.4 , 40.4	341
	43.4 , 43.4	295			40.4 , 40.4	341
	42.1 , 40.7	325			43.6 , 40.4	315
	39.4 , 39.4	358			38.7 , 38.7	371

	39.4 , 39.4	358			38.7 , 38.7	371
	39.4 , 39.4	358			38.7 , 38.7	371
	40.8 40.8	334			41.1 , 41.1	329
	40.8 , 40.8	334			38.6 , 38.6	373
	40.8 , 40.8	334			48.9 , 43.9	258

11 AFTER	Y	VHV		12 AFTER	Y	VHV
Rely X luting 2	37.7 , 37.7	396		Rely X luting 2	40.8 , 40.8	334
	41.7 , 41.7	320			42.5 , 42.5	308
	38.3 , 38.3	379			45.7 , 45.7	266
	38.3 , 38.3	379			41.6 , 38.9	344
	38.3 , 41.0	355			43.4 43.4	295

	41.0 , 39.5	344			43,4 ,41.4	309
	39.9 , 40.4	349			41.4 , 41.4	325
	36.0 , 36.0	429			40.1 , 40.11	346
	40.4 , 40.4	341			40.1 , 40.1	346
	38.3 , 38.3	379			40.1 , 40.1	346

13 AFTER	Y	VHV		14	Y	VHV
Rely X luting 2	29.0 , 36.2	523		Rely X luting 2	39.5 , 39.5	357
	36.2 , 36.2	425			39.5 , 39.5	357
	47.0 , 41.0	287			39.5 , 39.5	357
	42.9 , 39.2	331			39.5 , 39.5	357
	39.2 , 39.2	362			39.5 , 39.5	357

	39.2 , 40.0	355			39.5 , 39.5	357
	40.0 , 40.0	348			39.5 , 39.5	357
	40.0 ,40.0	348			39.5 , 39.5	357
	46.1 , 39.3	305			39.5 , 39.5	357
	39.3 , 39.3	360			40.8 , 40.8	334

15 AFTER	Y	VHV		16 AFTER	Y	VHV
Rely X luting 2	40.8 , 43.3	315		Transbond XT	46.1 , 46.1	262
	43.3 , 43.3	297			46.3 , 53.5	223
	40.9 , 44.7	304			53.5 , 53.5	194
	40.9 , 40.9	333			53.5 , 53.5	194
	39.2 , 39.2	362			48.3 , 48.3	238

	39.2 , 37.1	383			43,0 ,43.0	301
	41.6 , 43.3	309			43.4 , 43.4	295
	43.3 , 43.3	297			45.4 , 45.4	264
	41.3 , 41.3	326			48.7 , 48.7	235
	42.6 , 42.6	307			53.4 , 53.4	195

17 AFTER	Y	VHV		18 AFTER	Y	VHV
Transbond XT	37.2 , 37.2	402		Transbond XT	58.7 , 58.7	161
	38.1 , 38.1	383			56.7 , 56.7	173
	38.1 , 38.1	383			44.5 , 50.3	248
	38.1 , 38.1	383			55.1 , 55.1	183
	38.1 , 38.1	383			55.1 , 55.1	183
	38.1 , 38.1	383			55.1 , 55.1	183

	38.1 , 38.1	383			55.1 , 55.1	183
	38.1 , 38.1	383			55.1 , 47.5	211
	38.1 , 38.1	383			50.9 , 50.9	215
	38.1 , 38.1	383			48.2 , 48.2	239

19 AFTER	Y	VHV		20 AFTER	Y	VHV
Transbond XT	53.1 , 49.6	211		Transbond XT	35.0 , 35.0	454
	50.5 , 50.5				53.7 , 43.5	236
	52.7 , 51.7	204			39.2 , 41.5	343
	53.1 , 49.0	214			53.2 , 52.0	201
	68.9 , 64.9	124			63.4 , 58.6	150
	49.4 , 49,4	230			53.1 , 53.1	197

	51.6 , 50.5	214			55.5 , 50.0	200
	52.2 , 55.2	193			33.9 , 35.5	462
	46.5 , 45.5	263			43.3 , 44.0	293
	57.7 , 57.7	167			38.1 , 38.1	383

21 AFTER	Y	VHV		22 AFTER	Y	VHV
Transbond XT	48.2 , 57.9	198		Transbond XT	50.8 , 54.9	200
	47.4 , 47.4	248			48.6 , 48.6	236
	43.0 , 45.3	286			48.9 , 49.7	229
	46.0 , 48.9	248			52.7 , 52.7	208
	57.7 , 49.9	192			49.7 , 49.7	225
	68.0 , 55.9	145			45.9 , 46.1	263
	65.0 , 65.0	132			46.1 , 50.8	237

	74.3 , 68.5	109			50.4 , 50.4	219
	108.9 , 81.8	61			47.3 , 47.3	249
	76.2 , 65.4	111			50.4 , 46.5	237