MICROLEAKAGE AND MARGINAL ADAPTATION OF ULTRASONICALLY CURED GLASS-IONOMER SANDWICH RESTORATIONS

by

JEANINE FOURIE

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

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JEANINE FOURIE

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UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

DECLARATION

I, Jeanine Fourie, hereby declare that the work on which this dissertation is based, is original and neither the whole work, nor any part of it has been, is being, or shall be submitted for another degree at this or any other university, institution of tertiary education or examining body.

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JEANINE FOURIE

July 2008

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SUMMARY

Resin based composite is currently one of the most popular dental restoratives. Used as a direct restorative material, it displays many beneficial properties such as excellent micromechanical bonding to enamel, polishability and aesthetics. Despite many advances in dentine bonding agents, dentine bonding remains problematic with microleakage and recurrent caries, being frequent clinical sequelae.

The open sandwich technique was developed to overcome two problems: deficient bonding of resin composites to dentine, and inadequate strength and fracture toughness of conventional glass-ionomers (GI). GI displayed excellent cavity sealing abilities by virtue of their chemical adhesion to tooth structure. Resin-modified glass-ionomers (RMGI) were developed to improve on the weaknesses of conventional GI during early setting i.e. setting rate, water sensitivity and strength. Recently literature has reported the use of ultrasonic activation to set conventional GI, opening the possibility of improving the initial properties of the material and suitability for use in the open sandwich technique.

The aim of this study was to compare microleakage of Ketac Molar, Ketac Molar set by ultrasound (US), Vitremer and Ketac N100 used in the open sandwich technique, with the control of a resin based composite, Filtek Z250. Two hundred Class II cavities were prepared in a hundred caries free, human, molar teeth, with half of the cervical margins placed apical and the rest coronal to the cemento-enamel junction. For each material, twenty restorations were placed for each cervical position. The sandwich materials

were placed to fill the interproximal box level with the pulpal floor, and a final two layers of resin composite was then placed to complete the restoration.

Restored teeth were stored in a laboratory oven for 7 days at 37 ℃; margins were then finished initially with a medium grit Sof-Lex disc and finally with a fine diamond drill. Material groups were separated into two halfs to commence microleakage testing or thermocycling. Thermocycling was conducted for 500 cycles between 5 ℃ and 55 ℃, with a dwell time of 30 seconds.

Restored teeth were then covered with nail varnish around the restoration margins, and immersed in 0.5% basic fuchsin solution for 24 hours. They were then cleaned, embedded in clear self-curing acrylic and sectioned 3 times with an Accutom-2 precision saw, at 2 mm intervals. Sections were evaluated using a light microscope under 4 time's magnification and microleakage scores given as: 0 = no leakage; 1 = < ½; 2 = > ½ distance to the axial wall/pulpal floor; 3 = leakage up to axial wall/pulpal floor.

Statistical analysis was undertaken using Analysis of Variance (ANOVA) for the cervical and occlusal microleakage scores; *p*-values <0.05 were considered significant.

The cervical microleakage results of cavity margins in dentine showed that Ketac Molar (US) performed better than Ketac Molar, and Ketac N100 performed better than Vitremer. Results in enamel showed no significant differences. The use of the open sandwich technique effectively reduced microleakage of cervical cavity margins placed in dentine but failed to reduce occlusal microleakage of Filtek Z250.

OPSOMMING

Hars gebaseerde komposiet is huidiglik een van die mees gewilde herstellings-materiale. Dit het talle voordelige eienskappe as direkte herstellings-materiaal, o.a. uitstekende mikromeganiese binding aan glasuur, poleerbaarheid en estese. Ten spyte van die verbetering van dentien bindings-agense, bly dentien binding egter onbevredigend en lei dit dikwels tot mikrolekkasie en wederkerige karies.

In die intermediêre tegniek is konvensionele glas-ionomeer (GI) gebruik om die interproksimale boks te herstel om sodoende die gebrekkige dentien binding van komposiet te oorkom, terwyl komposiet in ‘n laag bo-oor die GI aangewend is om vir die swak meganies eienskappe van die GI te vergoed. Hars-gemodifiseerde glas-ionomeer (RMGI) verbeter op die tekortkominge van die GI, nl. verhardings tempo, vog sensitiwiteit en sterkte. Ultrasoniese aktivering kan ook gebruik word om GI te verhard, wat moontlik die aanvanklike gebrekkige eienskappe van die GI kan verbeter en dit meer geskik maak vir gebruik in die intermediêre tegniek.

Die doel van die studie was om mikrolekkasie te vergelyk tussen Ketac Molar, Ketac Molar verhard deur ultrasoniese aktivering (US), Ketac N100 en Vitremer gebruik in die intermediêre tegniek, met die kontrole Filtek Z250, ‘n hars komposiet. Twee honderd Klas II kaviteite is voorberei in ‘n honderd, gesonde, menslike tande, met die helfte van die servikale randte apikaal van die glasuur-sementale aansluiting. Vir elke materiaal is twintig herstellings geplaas vir beide servikale posisies. In die intermediêre tegniek is die material

geplaas in die interproksimale boks to op die vlak van die pulpale vloer, daarna is nog twee lae komposiet aangewend om die herstelling te voltooi.

Herstelde tande is gestoor vir 7 dae by 37 ℃ in ‘n laboratorium oond, waarna herstellingsrandte aanvanklik afgewerk is met medium growwe Sof-Lex skyfies en daarna met fyn diamante bore. Materiaal groepe is nou in twee verdeel om voort te gaan met mikrolekkasie toetse of termiese-siklering. Termiese-siklering is uitgevoer vir 500 siklusse, tussen 5 ℃ and 55 ℃, met ‘n dompelings tyd van 30 sekondes.

Naellak is rondom die kaviteits grense aangewend, en die tande in 0.5 % basiese fuchsin oplossing gedompel vir 24 uur. Daarna is monsters skoongemaak en in helder selfverhardende akriel gebed. Drie snitte is van die monsters gemaak, 2 mm van mekaar, met ‘n Accutom-2 presisie-saag. ‘n Ligmikroskoop is gebruik om snitte te evalueer, punte is toegeken as: 0 = geen mikrolekkasie; 1 = < ½; 2 = > ½ van die afstand na die aksiale wand/pulpale vloer; 3 = mikrolekkasie langs aksiale wand/pulpale vloer.

‘n Afsonderlike ANOVA satistiese analise is gedoen vir die okklusale en servikale mikrolekkasie waardes, waar ‘n *p*-waarde van <0.05 betekenisvol geag is.

Die resultate het getoon dat Ketac Molar (US) beter gevaar het as Ketac Molar, en Ketac N100 beter gevaar het as Vitremer wanneer servikale grense in dentien geleë is. Die gebruik van die intermediêre tegniek het mikrolekkasie aansienlik verminder wanneer servikale grense in dentien geplaas was, maar het nie die okklusale mikrolekkasie van Filtek Z250 verbeter nie.

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CHAPTER 1: LITERATURE REVIEW

1.1 INTRODUCTION: SANDWICH RESTORATIONS

McLean and Wilson (1977) first described the use of the sandwich technique to improve adhesion of resin composite restorations, for bonding composite resins to dentine (McLean *et al*, 1985) and for use in a tunnel preparation (McLean, 1987). The technique was developed to extend the limitations of posterior composite restorations, particularly the lack of permanent adhesion to dentine which could cause microleakage and postoperative sensitivity. According to McLean's suggestions, the cavity is completely filled with glass-ionomer (GI), where after the enamel walls are cleaned and the enamel and GI is etched with phosphoric acid followed with a dentine bonding agent and filled with a resin composite. Mount (1990) advocated that the GI at the dentine margin be left exposed to allow released fluoride to protect the surrounding tooth structure.

Many of the physical properties of the conventional GIs make them ideal for use in the sandwich technique. These properties include the fact that the materials release fluoride (Swartz, Phillips and Clark, 1984) and therefore have the potential for cariostatic activity (Francci *et al.,* 1999). Setting GI allow much more elastic compliance to volume reduction than resin composites, (Davidson, 1998), they bond to enamel and dentine (Wilson, Prosser and Powis, 1983) and show low setting shrinkage (Feilzer, De Gee and Davidson, 1988). GI can also be used in close proximity to the pulp but it is not desirable to place the materials in direct contact with the pulp (Hume and Mount, 1988). When a layer of dentine is present over the pulp the restorative and lining

materials are only mildly irritating to pulpal tissue (Pameijer, Segal and Richardson, 1981).

However the concept failed clinically when conventional glass-ionomers were used to restore the cervical margins of Class II restorations, mainly because of the continuous loss of material (Welburry and Murray, 1990; Knibbs, 1992; van Dijken, 1994; Yap, Mok and Pearson, 1997).

The aim of this *in vitro* study was to determine the effect of thermocycling, cervical position and the use of different materials on the cervical microleakage of Class II open sandwich restorations. The hypothesis was that the use of an intermediate layer at the cervical margin would improve the marginal seal of resin composite restorations, irrespective of thermocycling, or cervical position.

1.2 DIRECT RESTORATIVE MATERIALS

1.2.1 Resin composite

1.2.1.1 Structure

Resin based composites are indicated for use in moderately-sized Class II restorations. Resin composites are composed of four major components: organic polymer matrix, inorganic filler particles, coupling agent and an initiator-accelerator system (Craig and Powers, 2002).

- *Organic polymer matrix (oligomers)*: The two most often used oligomers are dimethacrylates, such as 2, 2-bis [4(2-hydroxy-3 methacryloyloxy-propyloxy) phenyl] propane (Bis-GMA) and urethane dimethacrylate (UDMA). Oligomers contain reactive carbon double

bonds at each end that can polymerise with each other. The viscosity of these materials, however, is often so high that triethylene glycol dimethacrylate needs to be added to reduce the viscosity of the final product (Craig and Powers, 2002).

- *Fillers:* Dental composites are classified according to the shape, size and distribution of the filler. Microhybrid composites contain irregularly shaped glass (borosilicate glass, lithium of barium aluminium silicate, strontium or zinc glass) or quartz particles of uniform diameter. These fillers may constitute up to 60% to 70% of the composite by volume or 77% to 84% by weight (Craig and Powers, 2002).

- *Coupling agents*: Silanes are used to achieve a bond between the inorganic fillers and the organic oligomers. This helps to transfer stress that is applied to a composite from one particle to another, instead of through the weak polymer. The coupling mechanism involves hydrolysis of the methoxy groups with bound water on the reinforcing filler. The unsaturated carbon double bonds are available for polymerization with the matrix during setting (Craig, 1981).

- *Initiators and accelerators:* Camphoroquinone is added in amounts of between 0.2% and 1%. It acts by absorbing blue light with a wavelength of about 470 nm, which is further accelerated by the presence of an organic amine, to initiate polymerisation of light cured composites. An excited triplet state is produced when champhoroquinone absorbs light, which together with the amine;

produce ion radicals to initiate polymerisation (Craig and Powers, 2002).

- *Pigments and other components*: Inorganic oxides are added to create shades matching most variations in natural tooth colour. Ultraviolet absorbers minimize colour changes due to oxidation (Craig and Powers, 2002).

1.2.1.2 Bonding mechanism

1.2.1.2.1Bonding to enamel

Buonocore (1955) showed that when enamel is pre-treated by phosphoric acid, increased adhesion is obtained by the increase in surface area due to the acid etching action. The use of acids also increases the wettability of the surface, allowing a more intimate contact between the acrylic resin and enamel, thereby favouring adhesion which may protect against secondary caries.

1.2.1.2.2Bonding to dentine

Bonding to dentine is inherently more difficult than bonding to enamel due to the organic nature of dentine. Dentine contains up to 22% water per volume and many resins are hydrophobic (Erickson, 1987). By removing the smear layer and smear plugs by the application of an acid, the dentinal tubules are opened allowing for the movement of fluid through the tubules because of the positive pulpal pressure (Brannstrom, 1986). The smear layer, which is left on the surface of dentine after instrumentation, should be removed because of its limited strength (Pashley and Carvalho, 1997). The structure of dentine also

varies tremendously between different sites of the tooth, with wider and more numerous tubules found closer to the pulp (Pashley, 1991). Buonocore, Wileman and Brudevold (1955) were the first to describe bonding to dentine, using an adhesive that was essentially a dimethacrylate with appended phosphate groups. These phosphate groups could chemically bond to the calcium in dentine, but bond strengths obtained with this bonding agent were very low and not hydrolytically stable. Dentine bonding systems evolved to the current era where systems consist of a variable grouping of etchant, primer and bonding agent. In the fourth generation adhesives these products are used separately to achieve bonding to dentine.

- *Etchant:* The acid etchant most often consists of 35% phosphoric acid, which removes the smear layer, and opens the dental tubules by removing the smear plugs and decalcifies the uppermost 10-15 µm of intertubular and peritubular dentine. By removing the mineral content, a dense network of collagen is exposed (Burke, Combe and Douglas, 2000). The total etch technique, where dentine and enamel are simultaneously etched, is commonly used. This technique initially lead to speculation regarding possible pulpal damage and hypersensitivity, but Kanca (1990) believed that sensitivity experienced is rather due to the inability of the subsequently placed restorative system to absolutely seal the restorative interface.

- *Primer:* The primer acts by penetrating the collagen network exposed by the etchant (Munksgaard and Asmussen, 1984). The primer has bifunctional molecular groups: the hydrophilic groups have an affinity

for wet dentine and the polymerisable groups can form carbon double bonds with the restorative resin. Formulations that contain 2-hydroxyethyl methacrylate (HEMA) are hydrophilic by nature of the hydroxyl group (Burke, Combe and Douglas, 2000).

- *Adhesive*: bis-GMA and other dimethacrylate resins are used in the adhesive component to penetrate the primed dentine. The combination of primed dentine and resin is termed the hybrid layer (Burke, Combe and Douglas, 2000).

The hybrid layer provides micromechanical bonding of resin to dentine, but resin tag formation may also contribute to the overall bond strength, especially close to the pulp where there are more dentinal tubules (Burke, Combe and Douglas, 2000). The dentine surface should be left moist after rinsing the etchant off to prevent collapse of the collagen network. Bonding systems that contain hydrophilic monomers such as HEMA are more tolerant of moisture, but excess moisture could interfere with bond quality (Tay, Gwinnett and Wei, 1996).

1.2.1.3 Polymerisation

During polymerisation reactive carbon double bonds polymerise with each other, reducing the bulk volume of the material. Polymerisation shrinkage can break the bond between the resin composite restoration and the tooth, producing a gap when gingival margins are placed in dentine (Jorgensen and Hisamitsu, 1984; Carvalho *et al.,* 1996; Wilson, Dunne and Gainsford, 1997). The flow characteristics of the material during early setting will determine the stress placed on the bonding interface by the contracting resin (Feilzer, De

Gee and Davidson, 1988). During pre-gel polymerisation the composite can flow which relieves stress within the structure (Davidson, De Gee and Feilzer, 1984), but as the material continues to polymerise, stress is placed on the composite-tooth bond. Techniques proposed to eliminate leakage due to polymerisation shrinkage include the incremental placement of composite, clear matrices, reflective wedges, beta-quartz inserts, the use of auto polymerising composite, inclusion of a gingival floor retentive slot, pulsed/stepped curing and sandwich techniques incorporating GIs or other materials as an intermediate layer (Hagge *et al*., 2001).

The C-factor is defined as the ratio of bonded to not-bonded surface areas of a restoration. The higher the C-factor the higher the rate of shrinkage stress development, because high C-factors such as in Class II restorations, leads to decreased flow capacity (Feilzer, De Gee and Davidson, 1987). Incremental placement of resin composites can reduce the C-factor by limiting the number of bonded surfaces (Lutz, Krejci and Oldenburg, 1986; Lui *et al.,* 1987) and reduce microleakage (Neiva *et al.,* 1998). Feilzer, De Gee and Davidson (1987) proposed that the adhesive bond might fail because shrinkage stress surpasses the bond strength with dentine. This was demonstrated by increased gap formation with increasing C-factor cavity preparations in resin composite restorations (Wattanawongpitak *et al.,* 2006).

Delayed light curing techniques reduce overall polymerisation contraction stresses (Unterbrink and Muessner, 1995), but may not be sufficient to eliminate gap formation and subsequent microleakage (Hagge *et al*., 2001).

1.2.1.4 Curing lights

Quartz-tungsten-halogen lights are used to polymerise composites at peak wavelengths of 450 to 490 nm, and intensity of 400 to 800 mW/cm^2 (Craig and Powers, 2002). Lower curing light intensity decreases polymerisation shrinkage stress and microleakage, and improves marginal adaptation (Uno and Asmussen, 1991; Feilzer *et al.,* 1995a; Unterbrink and Muessner, 1995). It is, however, still important to ensure a proper cure in all parts of the restoration (Rueggeberg, Caughman and Curtis, 1994). A metal matrix band is more practical for establishing the interproximal contour, but does not allow for a sufficient curing light intensity in the most apical portion of a Class II cavity. The tri-cure mechanism of Vitremer might overcome this problem when used in the sandwich technique (Dietrich *et al.,* 1999) because the acid base reaction and chemical cure continues in areas where the curing light fails to reach.

1.2.1.5 Clinical problems

The clinical success of posterior composite restorations is limited with respect to leakage (Davidson, Abdalla and De Gee, 1993), wear (Braem, Lambrechts and Vanherle, 1994; De Gee *et al.,* 1996) and longevity (Bernardo *et al.,* 2007; Raj, Macedo and Ritter, 2007). The restoration of deep approximal cavities needs to overcome the problems of difficult placement of a rubberdam, time consuming incremental technique and difficult handling of some dentine bonding systems (Friedl *et al.*, 1997).

Newer condensable composites have been brought on the market with the anticipated benefits of decreased wear, improved packability, increased depth

of cure, and reduced polymerisation shrinkage through increased filler loading. However, the accompanying high viscosity and modulus of elasticity brings with it limited wetting of the cavity walls during placement. To address this shortcoming an intermediate layer of restorative material has been suggested to improve marginal integrity and adaptation of the materials (Hagge *et al.,* 2001). Restoratives advocated for this purpose include flowable composites, flowable compomers, autopolymerising composites and RMGIs (Farah, Orton and Collard, 1998). The benefits of using GIs and RMGIs as intermediate restorative materials include the long term molecular bond to dentine and enamel, bacteriostatic action, thermal expansion similar to enamel and dentine and a slow setting reaction with low shrinkage (Mount, 1994a).

1.2.1.6 Microleakage

Dentine bonding agents are constantly improved upon, but the latest technology still fails to eliminate microleakage (Dietschi *et al.,* 1995; Hilton, Schwartz and Ferracane, 1997) and is not able to ensure a long-term hermetic seal (Van Meerbeek *et al*., 1998). Resin composites often present difficulties when used directly in posterior restorations (Roulet, 1997), and especially when cervical margins are placed in dentine (Hilton, Schwartz and Ferracane, 1997). In a study by Dietrich *et al,* (2000), Z100/Scotchbond 1 restorations showed severe microleakage on contaminated dentine that did not improve towards the deeper parts of the cavity.

Ferrari and Davidson (1996) compared microleakage of Class II restorations placed *in vivo* and *in vitro;* cervical and occlusal margins restored *in vivo*

showed more microleakage and air bubbles. In fact, microleakage free restorations could only be placed *in vivo* when no adjacent tooth was present because a greater degree of technical difficulty is associated with the placement of posterior composite restorations in the interproximal area. *In vitro* samples might therefore be of limited value in predicting the clinical performance of a dentine bonding system in Class II restorations (Ferrari and Davidson, 1996).

Significant microleakage was shown by Kenyon, Frederickson and Hagge (2007) and others (Dietschi *et al.,* 1995; Opdam, Roeters and Burgersdijk, 1998; Aranha and Pimenta, 2004) for direct and indirect Class II resin restorations with margins placed apically to the CEJ. It is known that Class II adhesive restorations can be placed to an acceptable standard if the gingival margin is in sound enamel (Loguercio *et al*., 2004), but too often margins extend into dentine, especially during the replacement of failed restorations (Dietrich *et al.,* 1999). As enamel approaches the CEJ, it becomes increasingly aprismatic, and even 1.5 mm coronal to the CEJ, both bond quality and strength are equivalent to those achieved to dentine rather than to enamel (Hilton and Ferracane, 1999). Two micromechanical mechanisms are important in bonding to dentine: hybridisation of the conditioned dentine (Nakabayashi, Nakamura and Yasuda, 1991) and resin tag formation (Gwinnett, 1994). One third of the strength of resin bonding to dentine is attributed to resin tag formation (Retief *et al.,* 1992).

Class II composite restorations with margins in dentine suffer from microleakage mainly because of dimensional changes of the composite (Prati

et al., 1994). The shrinkable bulk of composite can be reduced by using sandwich restorations with RMGI as base material (Roulet, 1994).

Another explanation for increased microleakage in dentine is the insufficient penetration of the bonding agent into the demineralised dentine (Thonemann *et al.,* 1999). This may occur either as a consequence of the collapse of the collagen structure when dentine is desiccated or by inadequate saturation with resin monomers (primer dilution) in the presence of excess water (Pashley and Carvalho, 1997; Tay, Gwinnett and Wei, 1998). All these events may occur simultaneously in Class II cavities after acid conditioning, where some of the cavity walls may be extensively dried, but the internal angles of the preparation may retain excess water (Loguercio *et al*., 2002).

1.2.2 Glass-ionomers

1.2.2.1 Structure

Glass-ionomers are composed of a glass (fluoroaluminosilicate glass), polyacid (polycarboxylic acid), tartaric acid and water. The glass is often contained in the powder while the acids may be included either in the liquid formulation or freeze dried and added to the powder. The use of higher molecular weight polyacrylic acid result in improved physical and mechanical properties (Nicholson *et al.,* 1988)

1.2.2.2 Bonding mechanism

Conventional GIs adhere to enamel and dentine by ionic bonding with hydroxyapatite (Wilson, Prosser and Powis, 1983). Tooth adhesion is a dynamic ion-exchange process in which the polyalkenoic acid softens and infiltrates the hydroxyapatite structure where it displaces calcium and

phosphate ions out of the substrate and forms an intermediate adsorption layer of calcium- and aluminium-phosphates and –polyacrylates at the glass-ionomer-hydroxyapatite interface (Lin, McIntyre and Davidson 1992; Mount 1994b).

Conventional GI bond to enamel even in the presence of a smear layer, but surface conditioners have been found to improve the bond strength, even when conditioning with phosphoric acid (PA) at various concentrations. Poly-acrylic acid (PAA) has been shown to be an effective conditioner (Powis *et al.,* 1982) and to improve the bond between conventional GI and enamel. The role of the conditioner probably involves the effective removal of the smear layer and improved wetting of the tooth surface by GI (Akinmade and Nicholson, 1992). The conditioners might also produce micropores in the enamel surface that either contributes to an increased surface for chemical bonding or to micromechanical bonding through polymer penetration (Glasspoole, Erickson and Davidson, 2002). Some studies have shown that even when bonding to dentine, conditioning with acids do not diminish the bond strength but sometimes even improve it (Smith and Martin, 1992). Conditioners remove the mineral phase from dentine, so it could be expected that the bond strength would be severely reduced if calcium binding was the only mechanism of adhesion, but the bonding mechanism could also include diffusion of polymer into the demineralised dentine and tubules to form micromechanical bonds. If the smear layer was not removed, the GI can still penetrate the smear layer through a self-etching process and effect a bond to the underlying enamel. The difference becomes apparent during bond strength tests: in unconditioned samples, the fracture mode is predominantly

adhesive at the enamel margin, but with smear layer removal more cohesive failures occur within the GI. The low pH of the liquid phase of GI enables the material to reach the enamel surface and to withstand the buffering capacity of enamel (Glasspoole, Erickson and Davidson, 2002). GI also seems to be insensitive to moisture within the dentinal tubules (Pashley, 1991).

1.2.2.3 Setting reaction

Glass-ionomers set by chemical gelation resulting from the reaction between an acid (polyacrylic acid) and a base (alumino-silicate glass). The setting stages may be summarised as: the release and migration of glass ions after acid attack; ion binding of cations to polyanions; precipitation of salts resulting in gelation and hardening; and hydration of the salts to develop the strength of the material. The addition of tartaric acid has improved the manipulation of the material, extended the working time and increased the setting rate (Crisp, Lewis and Wilson, 1976; Hill and Wilson, 1988)

Mclean (1979) stresses the importance of correct dispensing, mixing, placement, setting and finishing procedures and recommends that early water contamination should be avoided by protecting the setting material from dehydration by using varnishes recommended by the manufacturers.

The formation of a silica matrix network developing after the initial stage of the GI setting reaction (Wasson and Nicholson, 1993) allows an increase in bond strength after 24 hours, and a continuous increase in the compressive strength over one year due to the maturation of the material (Crisp, Lewis and Wilson, 1976).

The initial setting stage of GI is capable of reducing contraction stresses by viscous flow of the material, increasing the likelihood that the bond with the cavity walls will form and survive during setting and proves the suitability of GI as a base material (Dauvillier *et al*., 2000).

The setting of GIs can be divided into two stages: a first friable and rubbery stage which gradually transits to a strong and brittle stage. The initial rubbery characteristics of GI allows more elastic and thus non-destructive compliance for volume reduction than the rigid resin composites, but the initial low cohesive strength, coupled with the shrinking volume can lead to micro-crack formation throughout the restoration (Davidson, 1998).

1.2.2.4 Physical properties

Glass-ionomers have low flexural strength, low fracture toughness and low abrasion resistance in areas of heavy occlusal stress (Wilson, 1989). Improvements in physical and mechanical properties were reported by reinforcement using a disperse phase such as aluminium oxide, titanium oxide, zirconium oxide or aluminium titanate (Wilson, 1989), but the strength and surface integrity of GIs remain major drawbacks (McLean, 1987).

1.2.2.5 Microleakage

The use of GIs in Class V restorations has proven their reliability for long-term adhesion to tooth structure (Matis, Cochran and Carlson, 1996; McLean, 1996) and its use in the open sandwich technique, delivered promising results (Reid *et al.,* 1994; Dietschi *et al.,* 1995; Neiva *et al.,* 1998; Dietrich *et al.,* 1999). But the slowly developing bond between the GI and dentine can be disrupted by the contraction forces of a polymerising composite (Reid *et a*l.,

1994) and lead to failure. Although the more favourable setting rate of the conventional GI also allow for additional molecular movement and conformational changes during setting to effectively reduce shrinkage stress (Gladys *et al*., 2001).

1.2.2.5.1Effect of desiccation on microleakage

Dehydration will cause the GI restoration to shrink and crack, subjecting the newly formed ionic exchange layer at the interface with the underlying tooth structure to stresses (Mount, 1990). Even mature restorations subjected to desiccation may be severely stressed and compromised by the resultant shrinkage (Watson, Billington and Williams, 1991; Bouschlicher, Vargas and Denehy, 1996). The contraction of GI under desiccation conditions is far greater than expansion by water sorption, although an increase in the time of maturation of the restorative material reduces the extent of this contraction (Wilson and Paddon, 1993). Therefore, inadvertently desiccating restorative materials prior to dye immersion can increase microleakage scores (Bouschlicher, Vargas and Denehy, 1996).

1.2.2.6 Bond between glass-ionomers and composite resins

The sandwich technique takes advantage of the adhesive properties and biocompatibility of GI and the superior surface and aesthetics of the composite resin. For the success of this technique there should be a reasonable bond between the GI cement and the composite resin (Hinoura, Suzuki and Onose, 1991)

Initially it was believed that etching the glass-ionomer surface was required for micromechanical retention between the glass-ionomer and resin composite (McLean *et al.,* 1985; Hinoura, Moore and Phillips, 1987; Mount, 1989a). Studies showed that etching the GI surface markedly increased the bond strength to the dentine bonding agent/ composite resin (Sneed and Looper, 1985; Hinoura Moore and Phillips, 1987; Welburry *et al.,* 1988). McLean *et al.,* (1985) advocated the use of 37% phosphoric acid etchant on the set GI for up to 60 seconds followed by a low-viscosity bonding agent. The matrix of the set GI cement dissolves in acid, resulting in a rough and porous surface of exposed glass particles (Fuss, Mount and Makinson, 1990); the bonding agent then penetrates into the porosities and hardens. Roughening by cutting or etching reduces the surface contact angle and improves the wettability of the bonding resins used (Hinoura, Moore and Phillips, 1987; Mount, 1989b). Thorough washing of the GI cement after etching also increases the bond strength (Hinoura, Moore and Phillips, 1987). In etched samples bond failure occurs cohesively in the cement; with the bond strength between an etched GI and a composite resin being stronger than the cohesive strength of the GI (Sneed and Looper, 1985).

Hinoura, Suzuki and Onose (1991) investigated the factors which could influence the bond strength between the two materials: the time at which the GI is etched after commencing the mix; duration of etching; using different GI materials; and the placement of an intermediate unfilled resin layer. It was found that a low-viscosity dentine bonding agent should be placed as quickly as possible, and that the pH of the bonding agent has a limited association.

By waiting 24 hours before etching, one could improve the bond strength but this is clinically impractical (Knight, McIntyre and Mulyani, 2006).

The ultimate determinant of the bond strength between conventional GI and composites is the low cohesive strength because failure occurs predominantly cohesively (Kerby and Knobloch, 1992). RMGI being stronger than conventional GI, show significantly higher shear bond strengths (Kerby and Knobloch, 1992, Fortin, Vargas and Swift, 1995), with the composite type not being a determinant (Fortin, Vargas and Swift, 1995).

Etching of the GI surface may however affect the integrity of the material (Papagiannoulis, Eilades and Iekka, 1990) and their inherent roughness may be sufficient for micromechanical adhesion (Mangum, Berry and Parikh, 1990). Etching might even be detrimental to the bond between RMGI and composite (Rusz *et al.,* 1992). The application of a HEMA containing primer and a HEMA/BisGMA unfilled resin can improve the bond by improving wetting of the RMGI surface (Chadwick and Woolford, 1993).

Regardless of the controversies surrounding adhesion between GI and composites, no clinical studies of sandwich restorations showed failure directly related to the composite/GI interface (Welburry and Murray, 1990; Knibbs 1992).

In the co-cure technique, the GI is allowed to set or to set partially, a RMGI (mixed double liquid to powder) is painted on and the first increment of composite resin placed over the it. Both materials are then light cured for 10 seconds. This technique eliminates several steps and results in higher bond strengths because of the greater chemical bond of the RMGI between the GI

and resin composite (Knight, McIntyre and Mulyani, 2006). RMGI bonding agents have been shown to provide predictable long-term bonds between tooth structure and composite resin (Tyas and Burrow, 2002).

1.2.3 Glass-ionomers activated by ultrasound

Conventional GI can be fast or 'command' set by the addition of external energy such as ultrasonic excitation. Kleverlaan, van Duinen and Feilzer (2004) investigated the mechanical properties and compressive strength of glass-ionomers that were either chemically cured, ultrasonically activated or heat cured and concluded that the mechanical properties of GIs significantly improved after ultrasound and heat curing. This study included SEM evaluation of the set materials, but could not show visual changes related to the percentage of voids or a change in packing of particles.

Towler *et al.,* (2001) compared the mechanical properties of ultrasonically cured GI, with chemically cured GI, and found that the ultrasonically cured material demonstrated increased hardness, a decrease in the soft surface layer and negligible creep at a significantly shorter time after placement. Creep displacement should decrease as the polymer becomes increasingly cross-linked with time as the curing process proceeds, therefore, the lack of creep in the ultrasonically treated GI group shows that the curing process may be fully or at least more completed immediately after ultrasonic activation.

Ultrasonic activation can affect the curing process by:

1. Promoting more intimate mixing of the polyacid and glass powder, increasing the contact between components (Towler *et al.,* 2001; Kleverlaan, van Duinen and Feilzer, 2004);

2. Kinetic energy of the ultrasonic wave may accelerate dissolution of the polyacid and the glass and allow more rapid diffusion of the ionic species through the liquid, increasing the cross-linking process (Towler *et al.,* 2001, Kleverlaan, van Duinen and Feilzer, 2004);

3. Temperature of the ultrasonic apparatus increases the reaction rate (Kleverlaan, van Duinen and Feilzer, 2004);

4. The increase in temperature can lead to evaporation of the liquid, thereby increasing the powder to liquid ration (Kleverlaan, van Duinen and Feilzer, 2004).

1.2.4 Resin-modified glass-ionomers

1.2.4.1 Structure

In its typical form, hybrid ionomers consist of polycarboxylic acid or a modified polyacrylic acid with curable methacrylate groups grafted into the polymer backbone, a photocurable monomer, ion-leachable glass and water (Nicholson and McLean, 1992).

In some formulations a small portion of the pendant carboxyl (COOH) groups of the polyacrylic acid have been modified with isocyanatoethyl methacrylate, which introduces unsaturated (vinyl) groups pendant on the polymer backbone. HEMA is then added as a co-solvent to make the mixture more water soluble, and being an unsaturated group it will polymerise and copolymerise with the modified polyacrylic acid. Photo initiators are added to the liquid to allow the unsaturated (vinyl) groups pendant on the polyacrylic acid backbone to polymerise under the action of light and to further cross-link

the cement matrix, increasing rigidity and making it less prone to crazing (McLean, 1992).

The final set material is a complex structure in which glass particles are sheathed in a matrix consisting of two networks – one derived from the glass-ionomer the other from the resin (Wilson, 1990; Mitra, 1991). With the dual setting systems of RMGI's, the resin reinforcement provides higher mechanical strength and higher bond strengths to tooth surfaces compared with conventional GI (Uno, Finger and Fritz, 1996; Irie and Suzuki, 1999a; Irie and Suzuki, 2000).

1.2.4.2 Bonding mechanism

1.2.4.2.1Bonding to tooth structure

RMGI hold the possibility of bonding to tooth structure by the same chemical based bond as conventional GI, but in addition also by a micro-mechanical bond similar to resin composites (Erickson and Glasspoole, 1994). The double adhesion mechanism mainly determines their retention and marginal sealing capacity (Gladys *et al*., 1998). RMGI have been used in combination with resin bonding systems because they contain resinous components (Fritz, Finger and Uno, 1996a; Pereira *et a*l., 1998). This could help to simplify the sandwich technique so that only one treatment product is needed (Dietrich *et al*., 2000), but it is not really necessary since the HEMA concentration in RMGI already allows for good wetting and additional micromechanical adhesion (Glasspoole, Erickson and Davidson, 2002). Although Vitremer primer did not significantly improve the bond strength to enamel because of an insufficient etching effect, it still improved wetting of the tooth surface, while

conditioning with PA and PAA improved the bond strengths of RMGIs by enabling micromechanical bonding to occur. Higher bond strengths of RMGI were correlated to their higher fracture strengths (Glasspoole, Erickson and Davidson, 2002).

1.2.4.2.2Morphological interface

An essential feature of micromechanical bonding is the formation of resin tags into the dentinal tubules as well as the formation of a hybrid layer in inter- and peritubular dentine (Abdalla, 2000). Some evidence of a micromechanical bond in the form of a thin hybrid-like structure was observed with Fuji II LC and other RMGI's (Friedl, Powers and Hiller, 1995). SEM examination of the material interfaces showed that the types of dentine pre-treatment defined the interfacial morphology between dentine and restorative material. No evidence of an ion exchange layer could be detected morphologically (Gladys *et al*., 1998).

With Fuji II LC the dentine surface was conditioned with Dentine Conditioner which contains 10% polyacrylic acid to superficially demineralise the dentine and expose the collagen fibril network to allow the hybrid layer (500 nm) to form, therefore a thin resin rich layer separated the restorative material from the underlying dentine (Davidson, Abdalla and De Gee, 1993; Gladys *et al*., 1998). The HEMA content of the RMGI and the cleanliness of the dentine surface (Davidson, Abdalla and De Gee, 1993) left after smear-layer removal, may improve the ionic bonds, increase the wetting ability of the material and enhance its penetration into the exposed collagen network (Friedl, Powers and Hiller, 1995; Abdalla, 2000).

Vitremer uses a primer containing maleic acid for conditioning the dentine surface that removes most of the smear-layer but not the dentinal smear plugs (Friedl, Powers and Hiller, 1995). The primer also contains a copolymer of polyacrylic acid, HEMA and photo initiators (3M ESPE technical product profile). Although the material makes direct contact with the dentine substrate, no hybrid like zone has been reported (Friedl, Powers and Hiller, 1995; Gladys *et al.,* 1998), but a primer-dentine interdiffusion zone is sometimes seen (Shono, 1995 as cited by Miyazaki *et al.,* 1998). The bond is therefore more related to the development of the physical characteristics than chemical interaction of the cement matrix (Miyazaki *et al*., 1998).

1.2.4.3 Setting reaction

During setting, hybrid ionomers undergo two types of reactions: an acid-base reaction between the glass particles and the polyalkenoic acid, as well as free radical or photo-chemical polymerisation on exposure to light (Mitra, 1994 as cited by Abdalla, 2000). Therefore, RMGI, have longer working times than GI, and command set when exposed to a curing light. This makes them easier to use and more resistant to early moisture contamination and fracture (Rusz *et al.,* 1992). The initial peak strength of RMGI is reached by 24 hours (Mitra, 1994 as cited by Abdalla, 2000).

Even though RMGI show stability in a wet environment (Mitra and Kedrowski, 1994), they take up substantially more water than resin composites. RMGI exhibit polymerisation shrinkage similar to resin composites, which starts 5 minutes after light curing, and continues for the next 24 hours (Attin *et al*., 1995). This shrinkage gives rise to contraction stress which can damage the

adhesive interface and create marginal gaps. Polymerisation shrinkage stress can be reduced by various compensating mechanisms (Davidson, De Gee and Feilzer, 1984). Immersion in water leads to hygroscopic expansion due to water sorption (Attin *et al*., 1995; Feilzer *et al.,* 1995b; McLean, 1996; Yap, 1996), which may partially compensate for shrinkage. Prolonged storage in water storage leads to a continuous water uptake, resulting in stress relief and development of a compressive stress (Feilzer *et al.,* 1995b).

1.2.4.4 Microleakage

RMGI have shown to improve the marginal seal and adaptation of direct Class II restorations when used in a sandwich technique, compared to base or total bond restorations (Friedl *et al*., 1997; Dietrich *et al.,* 1999). The open sandwich technique used with RMGI should be the preferred technique when the cervical margin approaches dentine (Hagge *et al.,* 2001), because it presents the lowest degree of microleakage compared with GI or resin composite (Loguercio *et al*., 2002).

Promising clinical results have been reported for the combination of Vitremer and Z100 (van Dijken *et al.,* 1998). The use of one increment of Vitremer in combination with a metal matrix is likely to result in higher flow compensation of shrinkage stress due to a lower curing light intensity (Feilzer *et al*., 1995a; Dietrich *et al*., 1999). Using a metal matrix further improved microleakage (Dietrich *et al*., 1999) by making it easier to manipulate the material (Hilton, Schwartz and Ferracane, 1997).

Dietrich *et al.,* (1999) proposed to acid-etch all enamel margins prior to the application of the Vitremer primer, but the technical difficulties in ensuring this,

makes it possible that the dentine might be accidentally etched. However, bonding of RMGI to dentine appears to be less sensitive to contamination with saliva, blood or etchant which is likely to occur in a situation where cervical margins extend close to the gingival margin (Momoi *et al*., 1997; Dietrich *et al.,* 2000). This is possibly explained by hygroscopic expansion of the RMGI which may compensate for initial debonding (Fritz, Finger and Uno, 1996a). Despite the difficulties of sealing a proximal dentine margin, the RMGI sandwich technique was found to be the most likely to give successful clinical results (Wibowo and Stockton, 2001).

RMGIs are able to reduce microleakage in high C-factor cavity preparations by virtue of their greater elasticity which leads to reduced contraction stresses within the composite. If the material to tooth bond remains intact, the final rigidity of the material may play a compensating role in coping with the remaining polymerisation contraction stress (Kemp-Scholte and Davidson, 1990). The underlying RMGI is able to absorb some of the polymerisation stresses of the setting composite resin, and reduce the configuration factor to a more favourable internal shape (Davidson, 1994; Carvalho *et al.,* 1996), while the intrinsic porosity of hand mixed RMGI can increase the inner free surface area to contribute to stress relief (Alster *et al*., 1992; Davidson, 1994).

1.2.4.5 Physical properties

RMGI might not be as strong as resin composites but they still have acceptable physical properties such as: sufficient compressive strength, good bond strength to dentine, and fluoride release equal to conventional GI (Momoi and McCabe, 1993). RMGI are also claimed to have improved

marginal seal, reduced gap formation by hygroscopic expansion (Sidhu, Sherriff and Watson, 1997; Irie and Suzuki, 2000) and improved bonding durability after storage in water (Fritz, Finger and Uno, 1996a; Fritz, Finger and Uno, 1996b; Irie and Suzuki, 1999b; Irie and Suzuki, 2000). They might therefore be useful as a base in sandwich restorations (Dauvillier *et al.*, 2000).

1.2.4.6 Bond between resin-modified glass-ionomers and composite resins

Unpolymerised HEMA on the surface of Vitremer increases the surface wettability of the bonding agent to increase the bond strength, while the unsaturated methacrylate pendants on the polyacid chain within the polymerised RMGI, may also form covalent bonds with the resin bonding agent (Kerby and Knobloch, 1992). Vitremer achieved the highest bond strengths to resin composite, probably because the tri-cure setting mechanism and composition better enable the material to chemically bond to composites and especially to composites from the same manufacturer. Based on these findings it was recommended that RMGI be used in the sandwich technique (Fortin, Vargas and Swift, 1995). Failure between the RMGI and resin composite was found to be either cohesive (Farah, Orton and Collard, 1998) or adhesive (Fortin, Vargas and Swift, 1995). When composite was placed immediately after light curing the RMGI the likelihood of achieving a chemical bond is at its highest level (Fortin, Vargas and Swift, 1995). Surface treating the hybrid ionomers with phosphoric acid did not significantly increase the bond strength, possibly because the high resin content made these materials less susceptible to etching (Tate, Friedl and Powers, 1996).

1.3 PERFORMANCE TESTING OF DENTAL MATERIALS

1.3.1 Microleakage at cavity margins around restorations

The ultimate success of a material is indicated by its longevity in the oral cavity, but since the initial *in vitro* screening of new materials does not always reveal their full limitations or potentials, clinical testing remains the ultimate proof of their effectiveness (Van Meerbeek *et al.,* 1994). It has been shown that *in vitro* investigations can reliably predict (Ferrari *et al*., 1993), overestimate (Barnes *et al.,* 1993) or underestimate (Ferrari and Davidson, 1996) *in vivo* microleakage. Therefore, there is no scientifically validated means of correlating the results of laboratory tests with the clinical outcome (Roulet, 1994; Sudsangiam and van Noort, 1999).

In the oral cavity, multiple and mutually interactive clinical variables related to the tooth substrate and to its immediate environment, co-determine the eventual clinical effectiveness of newly developed adhesive materials (Bayne *et al*., 1991; Van Meerbeek *et al*., 1994). Due to the rapid evolution in dental technology and the development of new restorative materials, adhesive materials are currently marketed without sufficient clinical testing (Maneenut and Tyas, 1995). While the effect of microleakage on restoration longevity is somewhat unknown, it is suggested that interfacial leakage at the cervical interface can induce staining, post-operative sensitivity and pulpal inflammation (Aranha and Pimenta, 2004).

1.4 STORAGE TIME AND MEDIUM OF EXTRACTED TEETH FOR RESEARCH PURPOSES

An adequate sample size of extracted teeth is necessary when conducting *in vitro* microleakage studies to distribute intrinsic variables among the teeth. Since the structure of dentine influences bonding it is important to preserve this structure during storage for performance testing, while also protecting the researchers from pathogens

A number of techniques and materials have been investigated for the purpose of disinfecting and preserving the extracted tooth. Little difference in microleakage was seen between Class V restorations stored in ethanol, chloramine, thymol or restorations placed in freshly extracted teeth (Haller *et al.,* 1993). Thymol was, however, not recommended as storage solution due to the phenolic compound that could inhibit polymerization of methacrylates (Fujisawa and Kadoma, 1992; Haller *et al.,* 1993). Teeth should rather be removed from the thymol solution 24 hours before cutting of the cavities and be placed in distilled water (Swift, Pawlus and Vargas, 1995). It was also found that the effect of 0.1% thymol on dye penetration is similar to the effect of phosphate-buffered saline (Ziskind *et al.,* 2003).

Although ethanol (70%), formalin (10%), distilled water and 0.02% thymol increased the permeability of dentine without affecting bond strengths; it is still advised that teeth should not be stored for more than six months (Goodis *et al.,* 1993). Alcohol storage solutions failed to eliminate a number of pathogens that contaminated teeth after extraction (Pagniano *et al.,* 1985).

Teeth stored in formalin lead to significantly less microleakage compared to restorations placed in freshly extracted teeth (Haller *et al*., 1993); this was postulated to be due to collagen crosslinking by formaldehyde that may prevent collapse of the collagen fibrin network after acid etching. Chloramine, however, has no adverse effect on dentinal collagen and results in microleakage patterns similar to freshly extracted teeth (Haller *et al*., 1993). The use of 0.1% cetylpyridinium chloride does not affect bond strength to enamel, but may increase dye penetration at the cervical margin (Ziskind *et al.,* 2003).

Treatment of teeth with 2% gluteraldehyde, 1% sodium hypochlorite or placement in an autoclaving had no effect on the morphology of either cut- or uncut enamel (Shaffer, Barkmeier and Gwinnett, 1985). DeWald (1997) recommended the use of autoclaving teeth without amalgam restorations, based on the fact that it did not affect dentine permeability (Pashley, Tao and Pashley, 1993). Cryopreservation of teeth did not affect microleakage of two dentine bonding agents placed in Class V cavity preparations (Camps *et al.,* 1996).

1.5 STORAGE TIME AFTER PLACEMENT OF RESTORATIONS

A literature review by Raskin *et al.,* (2001) showed that most studies store restored specimens for less than 24 hours at 37 ℃ in distilled water before thermocycling commenced. A number of studies even stored teeth for 7 days at 37 ℃ in saline (Friedl *et al*., 1997), tap water (Hagge *et al.,* 2001) or distilled water (Doerr, Hilton and Hermesch, 1996; Loguercio *et al*., 2002).

The timing of microleakage studies may be especially important for GI, since their aging mechanisms are rather complex: strengthening results from additional cross linking and build-up of a silica gel phase, whereas weakening may result from erosion and the plasticizing effect of water (Cattani-Lorente, Godin and Meyer, 1994). GI should be stored for at least 24 hours in water to allow interaction between the GI and the substrate to continue, thereby resulting in higher bond- and cohesive strengths (Irie and Suzuki, 2000).

Less gap formation was also seen when finishing and polishing procedures of restorations were delayed for 24 hours *in vitro* (Irie, Tjandrawinata and Suzuki, 2003), but an *in vivo* study by Matis *et al*., (1991) showed no difference after three years between immediate and delayed finishing of conventional GI restorations.

1.6 TECHNIQUES FOR THE EVALUATION OF MICROLEAKAGE UNDER RESTORATIONS

1.6.1 History of microleakage evaluation

Microleakage is used as a measure by which clinicians and researchers can predict the performance of restorative materials in the oral environment and is based on the assumption that no restorative material is perfectly adaptive or adhesive to tooth structure. An interfacial gap of 2-20 µm which allows the penetration of bacteria, inadequate physical properties of the restorative material, and improper restorative technique or procedures all contribute to microleakage (Bauer and Henson, 1984). It is believed that the longevity of dental restorations would be enhanced if a restoration-tooth interface inhibits the movement of bacteria and/or its toxins (Kidd, 1976). Based on this requirement, microleakage testing is often used for the primary evaluation of

new adhesive materials (Hilton, 2002). But the wide variety of materials and techniques used *in vitro* leads to confusion because they lack common parameters for comparison, for example: dye concentrations, immersion periods, temperature, chemical nature of tracers and even methods to evaluate or score microleakage tests (Pashley, 1990).

1.6.2 Materials used for microleakage testing

Methods of investigation rely on movement of a visible medium between the restoration and the cavity margin; materials and techniques such as visualization, air diffusers, dyes, isotopes, bacteria and caries have been used to demonstrate microleakage (Bauer and Henson, 1984).

According to Bauer and Henson (1984) basic fuchsin is easy to use, non-toxic, have low cost and delivers reproducible results, although it showed less microleakage than clinical studies. Despite the fact that basic fuchsin is the dye most often used, the lack of standardisation prevented meaningful comparisons to be made between studies (Raskin *et al.,* 2001). Staining with 0,5% basic fuchsin indicated the most leakage and was more consistent than 2.0% fluorescent dye, 1.5% Reactive Orange 14 and ^{45}Ca, which showed the most variation in microleakage scores (Cochran *et al*., 2004). The sequential immersion of restored teeth in dyes showed that rhodamine B detected more microleakage than ^{45}Ca, which in turn detected more microleakage than methylene blue, suggesting that the size of the tracer was not the only important factor influencing microleakage. The consensus of agreement between evaluators was the highest for methylene blue and the lowest for rhodamine B (de Almeida *et al*., 2003).

A number of variables determined the extent of dye penetration, such as the tracer's particle size, pH, concentration and diffusion coefficient, as well as the thickness of dentine, and the surface area of dentine available for diffusion (Pashley and Matthews, 1993). But Youngson *et al.,* (1998) noted no difference between four tracers despite a wide range in pH. Tracers may lead to the overestimation of microleakage because of the permeability of dentine tubules (Gale and Darvell, 1999).

Dye penetration studies comparing a material susceptible to dehydration, such as conventional GI, with a material less susceptible should be cautiously interpreted because dehydration easily occurs during testing procedures and influences the microleakage of materials (Doerr, Hilton and Hermesch, 1996).

1.6.3 Techniques used for microleakage testing

Gale and Darvell (1994) demonstrated that microleakage is a three dimensional phenomenon and that different locations and angles of sectioning might result in completely different penetration scores. A single section seems to be insufficient to detect reliably the maximum tracer penetration at the tooth restoration interface, while three sections may avoid underestimating microleakage (Hilton, Schwartz and Ferracane, 1997). Despite the limitations of the single sectioning technique, it remains widely employed, and is still used in 88% of microleakage studies (Raskin *et al.,* 2001). By making two sections, four surfaces become available for evaluation (Kenyon, Frederickson and Hagge, 2007).

Three dimensional evaluations seem to be even more effective, given that microleakage was not uniform along the interface (Gwinnett and Yu, 1995).

When silver nitrate was used as a dye and the entire restoration was removed, a greater extent of microleakage was seen although the technique was extremely time consuming (Hilton, Schwartz and Ferracane, 1997, Wibowo and Stockton, 2001). The sequential grinding technique was also considered to be more accurate (Gladys *et al.,* 2001), but this assessment of dye penetration was still only semi-quantitative as it did not discriminate between severe leakage in large gaps and minimal leakage in minute gaps (Dietrich *et al*., 2000).

In Class II cavities, a four point scoring system has been used to indicate the extent of cervical and occlusal penetration of methylene blue solution: 0 for no leakage; 1 for less than half the length of the cervical box/ enamel dentine junction; 2 for leakage along the entire length of cervical box/ leakage deeper than the enamel dentine junction; and 3 for leakage along the axial/ occlusal wall (Ferrari and Davidson, 1996; Loguercio *et al.,* 2002). Raskin *et al.,* (2003) also used this scale to determine the influence of the number of sections on reliability of in vitro microleakage evaluations. The axial wall can also be divided in half to add another measurement, but does not add to the sensitivity of the test because of the number of measurements between less than or more than half the distance of the axial wall (Kenyon, Frederickson and Hagge, 2007).

1.7 THERMOCYCLING OF TEETH

1.7.1 History of thermocycling

The microleakage of a number of restorative materials has been shown *in vitro* to be directly proportional to their coefficients of thermal expansion

(COTE) (Bullard, Leinfelder and Russell, 1988), with the COTE of tooth structure measured at 11 x 10^{-6}/℃ (Bauer and Henson, 1984). The ISO TR 11450 Standard (1994) indicates that a thermocycling regime comprised of 500 cycles in water between 5 ° and 55 ℃ is an appropriate artificial aging test (as cited in De Munck *et al,*. 2005). Intra oral thermal changes compromise the bond between restorative material and tooth structure and create the potential for microleakage (Crim and Garcia-Godoy, 1987). Despite the theoretical implications of thermocycling testing, thermal stressing of restoration interfaces is only of value when the initial bond is already known to be reliable, which is unfortunately not the case for most current restorative materials (Gale and Darvell, 1999).

1.7.2 Effect of thermocycling on microleakage

Conventional GIs have a COTE very similar to tooth structure (Graig and Powers, 2002), and has been cited as an important factor in the successful adhesion of this material to tooth structure (Gladys *et al.,* 1998). The addition of resin in the RMGI has increased the COTE of the material (Mitra, 1994 as cited by Abdalla, 2000). All tooth coloured restoratives show expansion on heating, except for the GI and RMGI which actually show contraction due to water loss (Sidhu, Carrick and McCabe, 2004).

Thermocycling had no effect on the microleakage of conventional GI or RMGI, and therefore have comparable sealing ability (Sidhu, 1992; Doerr, Hilton and Hermesch, 1996; Friedl *et al*., 1997). Although the COTE of RMGI differs from tooth structure, other factors such as water sorption and the use of unfilled resins may offset this mismatch (Doerr, Hilton and Hermesch, 1996).

The restorative material has to compensate for changes due to the mismatched dimensional behaviour of the restoration and the adjacent tooth structure during thermal and mechanical fluctuations, the inclusion of air bubbles in hand-mixed materials enabled it to expand (Davidson, 1998).

Thermocycling increased the amount of microleakage for Class V restorations restored with resin composite, although not significantly (Kubo *et al.,* 2004). Overall microleakage of enamel margins was significantly less than at the dentine margins, but increased with increasing thermo cycles (Wahab, Shaini and Morgano, 2003)

RESEARCH OBJECTIVES

The research objectives of this *in vitro* study are:

1. To compare the marginal microleakage between a hybrid composite, RMGI, a nano filled RMGI, a conventional GI and a GI set by means of ultrasonic activation in direct Class II open sandwich restorations.
2. To determine the effect of thermocycling on marginal microleakage of the tooth-restorative interface in composite and sandwich restorations.
3. To determine the effect of the positioning of the cervical margin relative to the CEJ on marginal microleakage.

RESEARCH HYPOTHESES

1. The ultrasonically cured GI is superior to the conventional GI.
2. The nano filled RMGI is superior to the traditional RMGI.
3. Thermocycling of samples will influence the microleakage of restorations significantly.
4. Positioning of the cervical margin apical to the CEJ will result in greater microleakage.

CHAPTER 2: MATERIALS AND EXPERIMENTAL PROCEDURES

2 MATERIALS

The materials used in this study include a resin composite bonded with a fourth generation dentine adhesive, a conventional GI set conventionally and by means of ultrasonic activation, a RMGI and a nano-filled RMGI. These materials were selected to obtain a representative sample of materials used in direct restorative dentistry and accepted for use in the open sandwich technique. The primary aim of this study was to compare a resin composite to members of the GI family of materials; therefore, a polyacid modified resin was not included due to its close similarity to a resin composite.

Materials were selected from the same manufacturer to exclude the possible effects of material incompatibility on microleakage values, and to simulate the ideal situation. Material manufacturers often use the same resin in all of their restorative materials and therefore lead to improved co-polymerisation.

2.1 RESIN COMPOSITE RESTORATIVE

2.1.1 Filtek Z250[1]

- Filtek Z250[1] (Lot 6WH) is a visible light-activated, radiopaque, restorative composite (Figure 1:). A dental adhesive is used to permanently bond the restoration to tooth structure.

- Resin: the majority of tri(ethylene glycol) dimethacrylate (TEGDMA) has been replaced with a blend of urethane dimethacrylate (UDMA)

[1] 3M ESPE, St Paul, MN, USA

and Bisphenol A polyethylene glycol diether dimethacrylate (Bis-EMA). Both of these resins are of high molecular weight and therefore have fewer double bonds per unit of weight. The higher molecular weight reduces the polymerisation shrinkage, imparts a greater hydrophobicity and is less sensitive to changes in atmospheric moisture.

- Filler: zirconia/silica filler is loaded 60 % by volume. The particle size distribution is 0.01 µm to 3.5 µm with an average particle size of 0.6 µm.

- This material is indicated for use in the sandwich technique with glass-ionomer resin material (3M Filtek™ Z250 Universal Restorative System Technical Product Profile/ Instructions for use).

2.1.2 ScotchBond Multi-Purpose Plus[1]

- This bonding agent is indicated for direct placement of light-cured composite (Figure 2).

- 35% Phosphoric acid (LOT 6HG) at pH 0.6 is used to etch the enamel and to remove the dentinal smear layer and uppermost intertubular dentine (Figure 3).

- The primer (LOT 7BH) at pH 3.3 is an aqueous solution of HEMA and a polyalkenoic acid copolymer which resist the detrimental effect of moisture in a relative high humidity environment. The primer allows the subsequently placed resin to “wet” the etched surface.

- The adhesive (LOT 7PW) is a combination of Bis-GMA and HEMA resin (3M Scotchbond™ Multi-Purpose Plus Technical Product Profile).

2.2 CONVENTIONAL GLASS-IONOMER

2.2.1 Ketac Molar Easymix[1]

- The powder (Lot 279629) consists of a very fine, radiopaque aluminium-calcium-lanthanum fluorosilicate glass. The particle size distribution shows that 90 % of all particles are smaller that 9 µm and 10% are smaller than 1 µm.

- The polycarboxylic acid is distributed to 60 % in the powder and 40 % in the liquid (Lot 275416).

The Ketac Molar Easymix[1] granulated powder has a significantly improved wettability to simplify mixing (3M Ketac™ Molar Easymix Technical Product Profile) (Figure 4).

2.2.2 Ketac Conditioner[1]

(Lot 258803)

A 25% polyacrylic acid solution for removal of the smear layer after tooth preparation (3M ESPE Ketac™ Conditioner: Instructions for use).

2.3 RESIN-MODIFIED GLASS-IONOMER

2.3.1 Vitremer Tri-Cure Glass-Ionomer System[1]

- Powder (LOT 7MY) is composed of a radiopaque, fluoroaluminosilicate glass, microencapsulated potassium persulfate and ascorbic acid

which provides the methacrylate cure of the glass-ionomer. Pigments provide shade colouring.

- The liquid (LOT 7FY) is a light sensitive, aqueous solution of a polycarboxylic acid modified with pendant methacrylate groups, water, HEMA and photo initiators.

- The primer (LOT 7BM) is a one part, visible light-cure liquid designed for use with the tri-cure glass-ionomer. It is composed of the Vitrebond[1] copolymer, HEMA, ethanol and photo initiators, similar to the Vitrebond[1] liquid. The function is to modify the smear layer and adequately wet the tooth surfaces to facilitate adhesion of the glass-ionomer (3M Vitremer™ Tri-Cure Glass-Ionomer System Technical Product Profile) (Figure 5).

2.3.2 Ketac N100[1]

Ketac N100[1] (Figure 6) is a new resin-modified glass-ionomer brought on the market by 3M ESPE. This product combines the traditional characteristics of the RMGI with bonded nanofiller technology. This results in a material with improved final polish and aesthetics. Ketac N100[1] is marketed as a two part paste, light cured RMGI direct restorative. The two part system is delivered in a multi-dose Clicker™ Dispenser.

The chemistry of Katac Nano[1] is based on the methacrylate modified polyalkenoic acid also used in Vitrebond[1]. This polyacid is capable of cross links via pendant methacrylate groups as well as the acid-base reaction

between the fluoroaluminosilicate glass and the acrylic and itaconic acid copolymer groups. Water is present to facilitate the ionomer reactions.

Two chemical reactions occur during the setting process of Ketac N100[1]. The first is the traditional glass-ionomer reaction between fluoroaluminosilicate glass and the polycarboxylic acid functional polymer in the presence of water. The other setting reaction is by polymerisation of methacrylate functional monomers, oligomers and polymers. Reactive double bonds are consumed during the polymerisation process.

The fillers consist of an acid reactive fluoroaluminosilicate glass (FAS) and a unique combination of nano fillers. The filler loading is approximately 69% by weight. All of the nano fillers are surface modified with methacrylate silane coupling agents to provide covalent bond formation into the free radically polymerised matrix. The nano fillers range between 5-25 nm in size and are non-agglomerated and non-aggregated. The fillers are derived from both silica and zirconia. The FAS glass is radiopaque, and has an approximate particle size of less than 3 microns.

- Two part paste system (Lot AB7AA):
    - Aqueous paste: acidic polyalkenoic acid, reactive resins and nano fillers.
    - Non aqueous paste: FAS glass, reactive resins and nano fillers.
- Filler (69%):
    - 27% FAS glass (acid and free radically reactive).

- 42% methacrylate functionalized nano fillers (acid and free radically reactive).

- Primer (Lot 7AA):

  - Visible light-cure liquid consisting of a methacrylate modified polyalkenoic acid, water and photo initiators.

  - The primer is acidic in nature, with the function of modifying the smear layer and to wet adequately the tooth surface to facilitate adhesion of the restorative.

(3M Ketac N100™ Light-Curing Nano-Ionomer Restorative. Technical Product Profile).

2.4 EXPERIMENTAL PROCEDURES

2.4.1 Mounting of teeth

Freshly extracted adult human molar teeth were cleaned with an ultrasonic scaler (NSK, Varios 350)[2], pumice and water. Teeth were collected from the Maxillo-Facial and Oral Surgery department at the Oral and Dental Hospital. Reason for extraction of the teeth was not disclosed. Permission to use the teeth was obtained from the Research Committee of the School of Dentistry, University of Pretoria, and the Decleration of Helsinki signed. From these teeth, one hundred sound molar teeth were selected following examination for cracks and caries with a light microscope (Nikon 33759)[3]. These teeth were

[2] NSK, Taito-ku, Tokyo, Japan

[3] Nikon, Tokyo, Japan

stored in 0.2 % thymol (Merck)[4] (Figure 7) at 4 ℃ until commencement of the study. The apexes of the teeth were embedded in self-curing acrylic (Excel Rapid repair)[5] with the long axis of the teeth oriented in a vertical position (Figure 8).

2.4.2 Cavity preparation and placement of matrix band

Two hundred Class II cavities were prepared mesially and distally in each molar tooth. Half of the cavities were prepared with the cervical margins placed 1 mm coronal to the cemento-enamel junction (CEJ) and the rest 1 mm apical to the CEJ so that each tooth had a cavity both in enamel and in dentine. A diamond dome fissure bur (ISO 314 141)[6] (Figure 9), in a fast hand piece, under water cooling was used to cut the cavities. The dimensions of the cavities were 5 mm bucco-lingually, a pulpal floor/ axial wall depth of 2 mm with rounded internal line angles (Figure 10). The burs were replaced after every ten cavity preparations with a new bur. Each of the cavities was washed for 15 seconds with water and lightly air dried with an air syringe. A Tofflemire matrix band[7] was used during placement of the restorative material to prevent gingival overhangs.

[4] Merck, Darmstadt, Germany

[5] Wright Health Group, Dundee, Tayside, Scotland

[6] Edenta, Hauptstrasse, Switzerland

[7] Hawe Neos Dental, Bioggio, Switzerland

2.4.3 Application of restorative materials

The one hundred teeth containing the cavity preparations were randomly divided into 5 groups of 20 each (Figure 11: n = number of restorations). The teeth were mixed in a container and randomly selected and assigned to a material. There was therefore no systematic selection procedure employed. One group was entirely restored with Filtek Z250[1] and Scotchbond Multi-Purpose Plus (SBMP)[1] as control. The rest of the teeth were restored with the open sandwich technique, where an intermediate layer of either Ketac Molar Easymix[1], Ketac Molar Easymix[1] with ultrasonic activation, Vitremer[1] or Ketac N100[1] was used to restore the interproximal box level with the pulpal floor, and the rest of the cavity restored with Filtek Z250. All restorative materials were applied according to the manufacturers' instructions.

2.4.3.1 Control: direct composite Filtek Z250[1]

Etchant was applied to the enamel surfaces for 15 seconds and to dentine surfaces for 10 seconds. The etchant was washed off for 5 seconds with water and the surface gently blotted dry with a cotton pellet. The SBMP[1] primer was applied to all cavity surfaces and gently air dried with the air syringe for 5 seconds. The adhesive was applied to the same surfaces and light cured with an Optolux 501[8] light curing unit for 10 seconds. A Tofflemire matrix band[7] was now applied to prevent gingival overhang of the restorative material. The first layer of Filtek Z250[1] was placed in the gingival box area up to a maximum thickness of 2 mm and light cured for 20 seconds. Two final

[8] Kerr Corporation, 1717 West Collins, Orange, California

layers were placed obliquely in the occlusal aspect of the cavities (Figure 12) and each light cured for 20 seconds.

2.4.3.2 Vitremer[1] sandwich restoration

All of the enamel walls were etched for 15 seconds and rinsed. The occlusal dentine was also etched for 10 seconds, rinsed for 5 seconds with water and the dentine blotted dry. Vitremer Primer[1] was applied to the axial wall and gingival step, left for 30 seconds then air dried and light cured for 20 seconds. A Tofflemire matrix band[7] was placed. Vitremer[1] was mixed in the ratio of 2 drops of liquid to 2 scoops of powder, and inserted by means of the dispensing tip into the proximal box to fill it level with the pulpal floor and light cured for 40 seconds. A ball burnisher wetted with the adhesive of SBMP[1] was used to adapt the material. SBMP[1] primer, adhesive and Filtek Z250[1] was now applied to the remaining cavity surfaces as for the control procedure.

2.4.3.3 Ketac N100[1] sandwich restoration

All of the enamel walls were etched for 15 seconds, and the pulpal floor was etched for 10 seconds, the entire cavity washed with water for 5 seconds and the dentine blotted dry with a cotton wool pellet. Ketac N100 Primer[1] was applied to the axial wall and gingival step, left to wet the surface for 15 seconds then air dried for 10 seconds and light cured for another 10 seconds. A Tofflemire matrix band[7] was placed. Equal quantities of Ketac N100[1] was dispensed with the clicker dispenser onto a waxed paper and mixed for 20 seconds. The mixture was then inserted by means of the dispensing tip into the proximal box to fill it level with the pulpal floor and light cured for 30 seconds. A ball burnisher wetted with the adhesive of SBMP[1] was used to

adapt the material. SBMP[1] primer, adhesive and Filtek Z250[1] was now applied to the remaining cavity surfaces as for the control procedure.

2.4.3.4 Ketac Molar Easymix[1] sandwich restoration

All of the enamel walls were etched for 15 seconds. The occlusal dentine was also etched for 10 seconds and the entire cavity rinsed for 5 seconds. Ketac Conditioner[1] was applied to the proximal box surface for 10 seconds, rinsed with water and the cavity blotted dry. A Tofflemire matrix band[7] was placed. Ketac Molar Easymix[1] was mixed in the ratio of one drop of liquid to one scoop of powder, and inserted into the proximal box to fill it level with the pulpal floor. A ball burnisher wetted with the adhesive of SBMP[1] was used to adapt the material, this layer of adhesive served the dual purpose of ease of manipulation and protecting the setting glass-ionomer from desiccation. The material was left for at least 5 minutes before continuing with the SBMP[1] primer, adhesive and Filtek Z250[1] for the remaining cavity surfaces as for the control.

2.4.3.5 Ketac Molar Easymix[1] activated by ultrasound sandwich restoration

The same procedure was followed as for the Ketac Molar Easymix[1] sandwich restoration, but instead of allowing the GI to set, an ultrasonic tip for amalgam condensation (NSK, Satelec G28[2])[2] (Figure 13) mounted in a NSK, Varios 350[2] scaler, was applied to the available surface of the glass-ionomer. The tip was applied without water for 30 seconds. The NSK[2] scaler was set at 'General', level 2, to operate at a frequency between 28 to 32 KHz. SBMP[1]

primer, adhesive and Filtek Z250[1] was now applied to the remaining cavity surfaces as for the control.

2.4.4 Storage of restored teeth

Restored teeth were stored in distilled water at 37℃ for 7 days (Friedl *et al*., 1997; Hagge *et al*., 2001; Doerr, Hilton and Hermesch, 1996 and Loguercio *et al*., 2002) in a temperature controlled Precision Scientific Company[9] laboratory oven. According to Irie and Suzuki (2000), 24 hours is sufficient to allow maturation of the GI bond.

2.4.5 Thermocycling of restored teeth

Half of the restored specimens (n 10) for each group of materials and cervical position were thermocycled in tap water for 500 cycles between 5 ℃ and 55 ℃ with a dwell time of 30 seconds. The thermocycling unit consists of two baths, the one bath at a temperature of 5 ℃ using a Labotec[10] cooling unit, and the other at 55 ℃ using a Büchi[11] water bath (Figure 14).

2.4.6 Finishing of restorations

Restorations were contoured with a fine diamond burr (ISO 011 0943) under water cooling and polished with medium grit Sof-Lex[1] (Figure 15) discs to remove any excess restorative material from the cavity margins.

[9] Precision Scientific, Mumbai, Maharashtra, India

[10] Labotech, Randjiespark, Midrand, South Africa

[11] Buchi, Uster, Switzerland

2.4.7 Treatment of specimens with basic fuchsin

After impressions were taken, all of the teeth were dried with paper towels and covered with nail varnish[12] up to 1 mm surrounding the cavity margins (Figure 16). The teeth were then immersed in a 0.5 % solution of basic fuchsin[4] (Figure 17) for 24 hours at 37 ℃. Care was taken not to leave specimens to dry out for any given period of time, except for the necessary procedures of impression taking and painting with nail varnish[15].

2.4.8 Sectioning of specimens

After 24 hours teeth were removed from the basic fuchsin[4] solution and rinsed under tap water. The nail varnish[17] was then removed from the teeth with hand scaling instruments[13]. All the teeth were now embedded in clear self-curing acrylic resin (Excel Rapid Repair)[5] (Figure 18). Three longitudinal sections of the embedded teeth were made 2 mm apart with an Accutom-2[14] (Figure 19) precision saw to yield 2 segments and four surfaces for evaluation.

2.4.9 Microleakage assessment of sectioned specimens

The penetration of basic fuchsin dye between the restoration and either the occlusal or cervical cavity margin was determined by examining sectioned samples under a light microscope. Microleakage scores were given for the cervical cavity margin as: 0 = no penetration (Figure 20), 1 = penetration less

[12] Revlon, New York, U.S.A

[13] Hu-Friedy, Chicago, IL, U.S.A.

[14] Struers A/S, Pederstrupvej 84, DK – 2750 Ballerup

than half the distance of the cervical step to the axial wall (Figure 21), 2 = more than half the distance (Figure 22) and 3 = up to and including the axial wall (Figure 23). The same scoring system was adapted for the determination of occlusal microleakage, this time using the distance between the occlusal cavity margin and the pulpal floor (Figure 24, Figure 25 and Figure 26). Stereomicroscope pictures were taken under magnification of: microscope 1.5; intermediate lens 0.5 and camera 2. The examiner performed the microleakage assessment twice using an Olympus[15], BH2 light microscope under 4 time's magnification. Microleakage values gained during the second assessment was used to calculate the microleakage scores.

2.4.10 Statistical analysis of data

The statistical model analysed the main effects (material differences, thermocycling and cervical position differences) and interaction effects (first and second order interaction between the factors). The model contained these effects in a multivariate linear additive form of a three-factor design, together with a random term (representing statistical variation) which can be assumed to have a normal distribution. The dependent (or response) variable is leakage and is analysed for the mentioned model in an analysis of variance (ANOVA), that was performed once for cervical and once for occlusal microleakage.

The data was captured in Excel format and imported to the statistical package SAS©. From the average microleakage data the least squares means (LS means) was calculated. Comparisons were made according to the LS means,

[15] Olympus, Shinjuku-ku, Tokyo, Japan

differences were deemed statistically significant when the *p* value was less than 0.05.

Figure 1: Filtek Z250

Figure 2: Scotchbond Multi-Purpose Plus

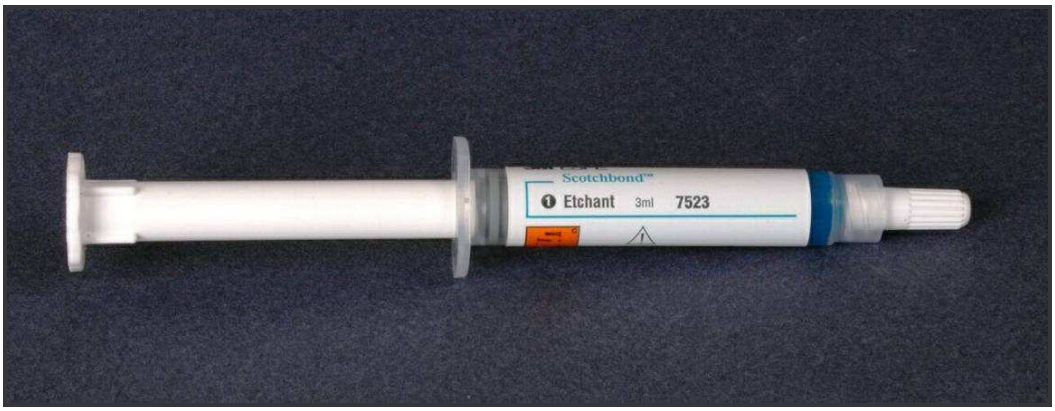

Figure 3: Scotchbond Etchant

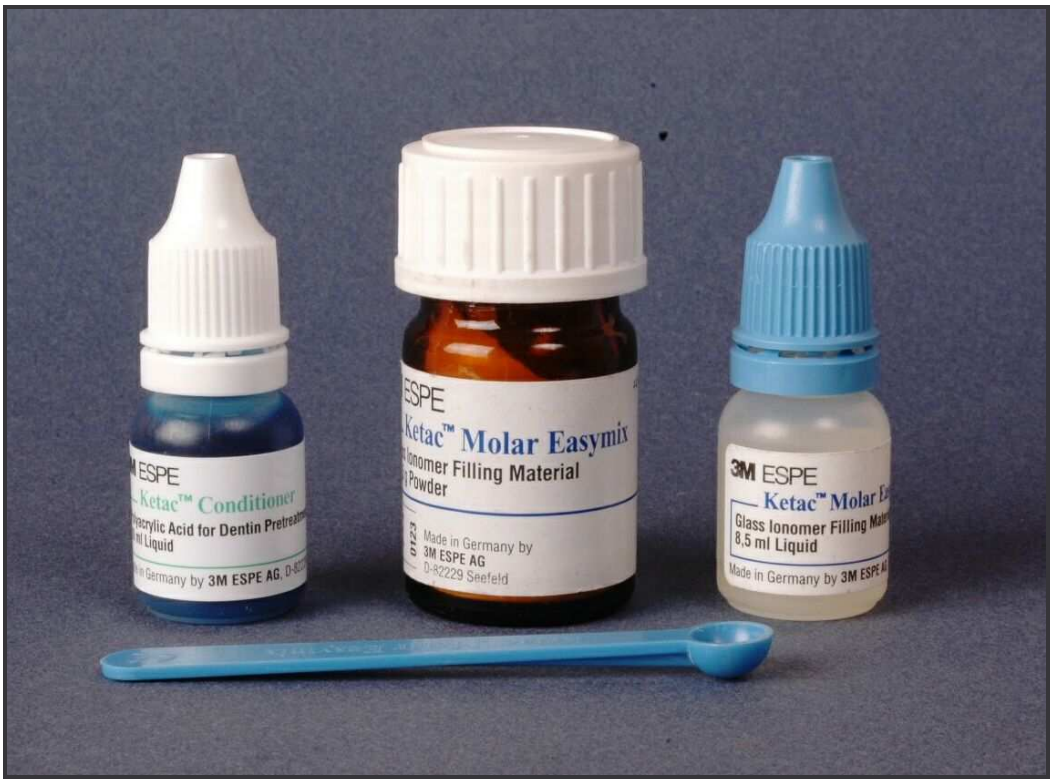

Figure 4: Ketac Molar Easymix

Figure 5: Vitremer

Figure 6: Ketac N100

Figure 7: Thymol

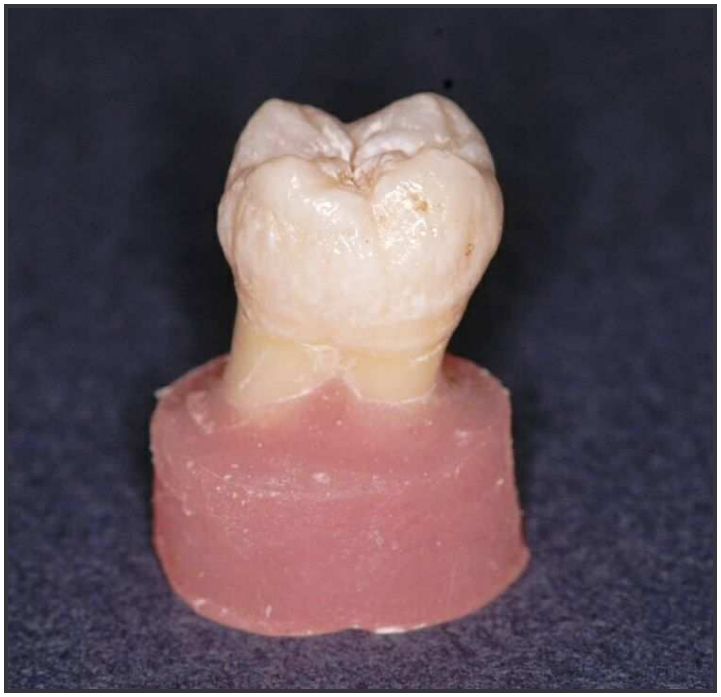

Figure 8: Caries free molar embedded with apices' in self curing acrylic

Figure 9: Edenta diamond bur

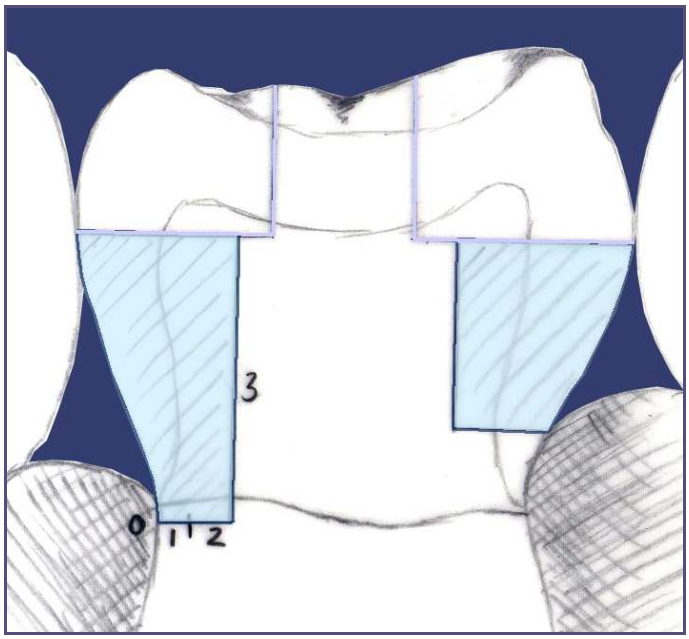

Figure 10: Line drawing of Class II cavity preparation in a molar tooth

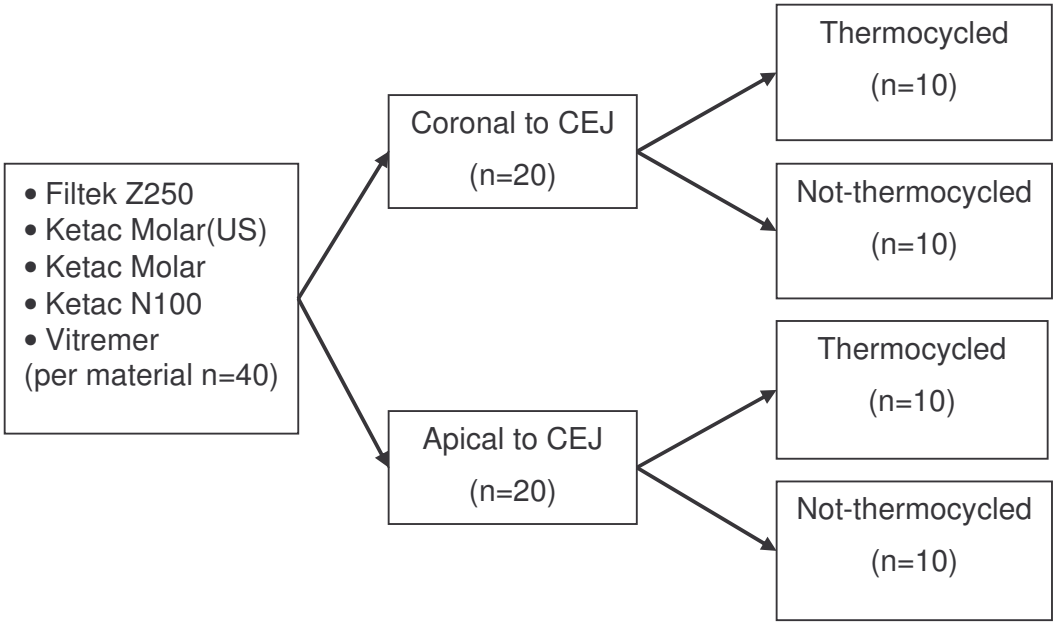

Figure 11: Flowchart of distribution of cavities between materials, cervical position and thermocycling

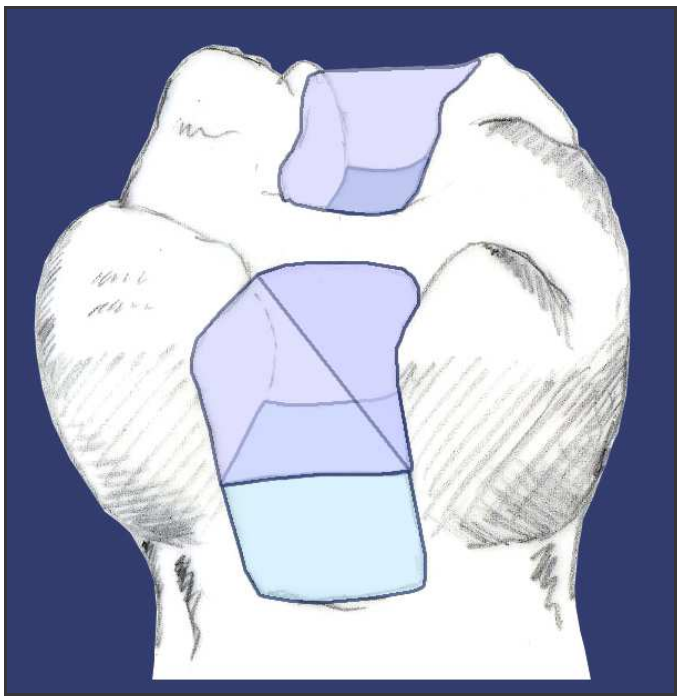

Figure 12: Line drawing indicating placement of restorative material

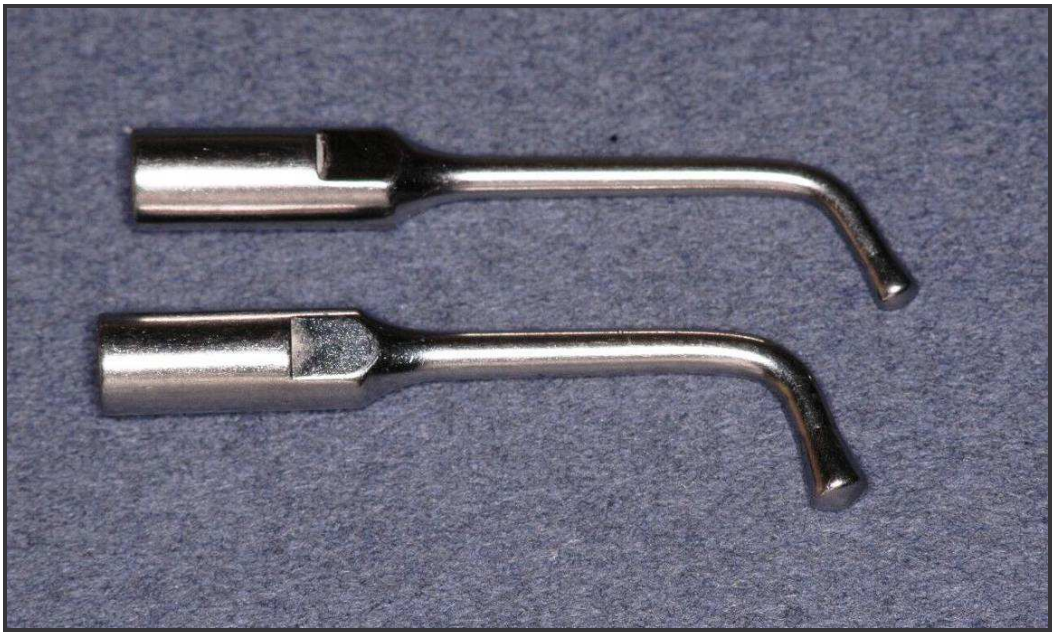

Figure 13: P5 Ultrasonic condensing tip

Figure 14: Thermocycling unit with warm and cold baths

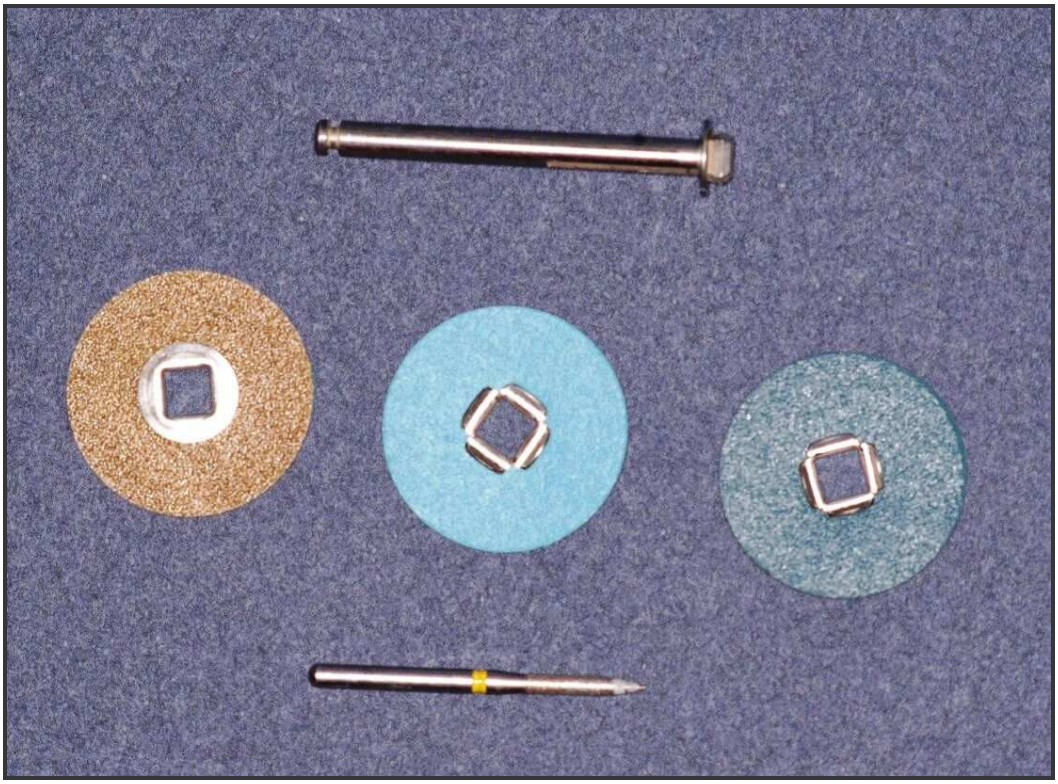

Figure 15: Sof-Lex disc with mandrel and diamond finishing bur

Figure 16: Human molar tooth painted with nail varnish

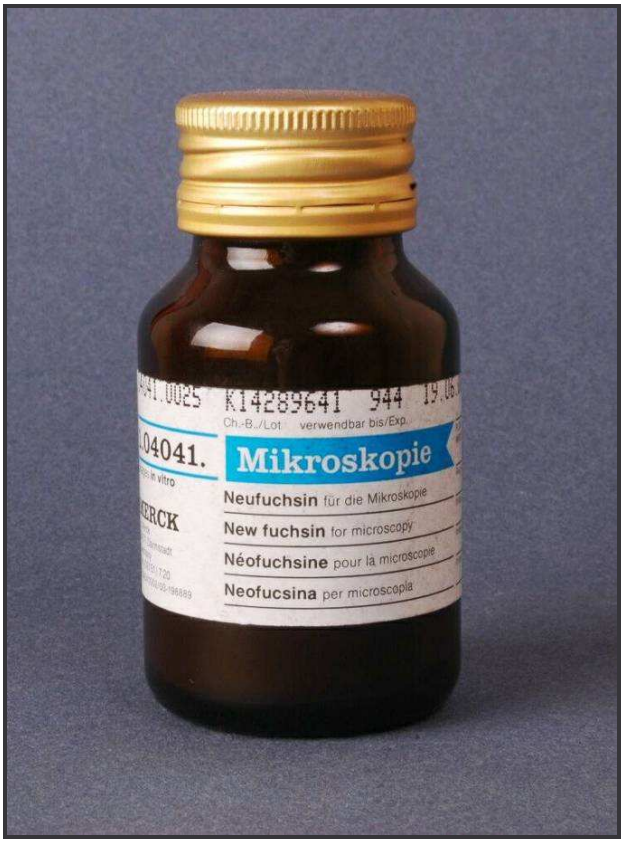

Figure 17: Basic Fuchsin

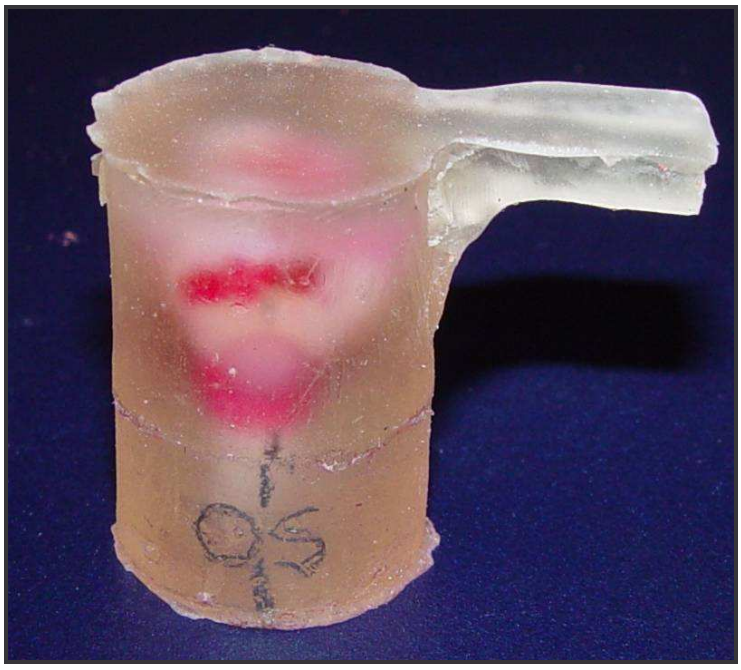

Figure 18: Tooth with Class II restoration embedded in self curing acrylic

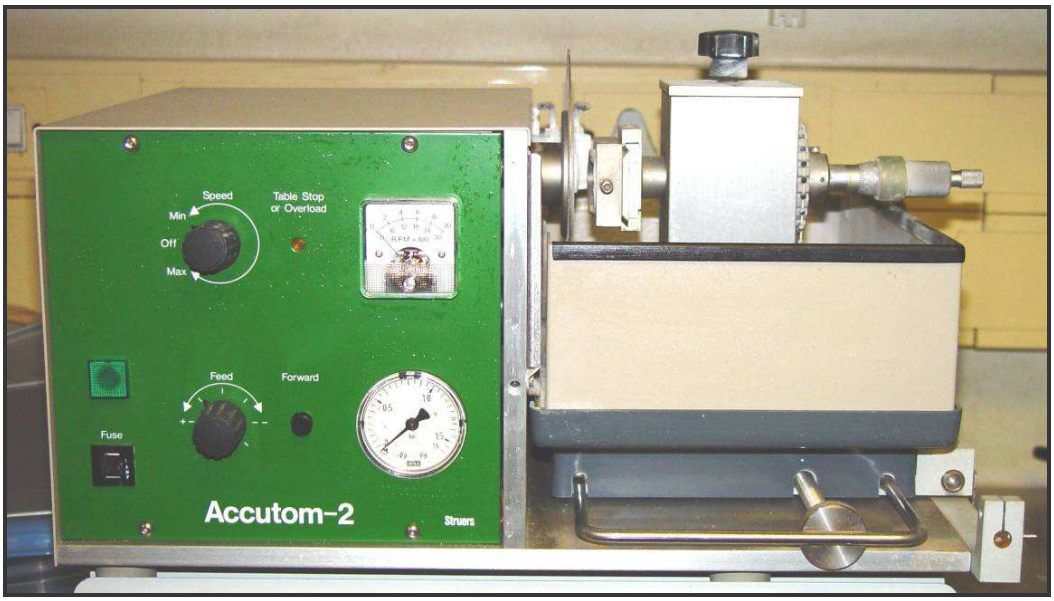

Figure 19: Accutom-2 Precision saw

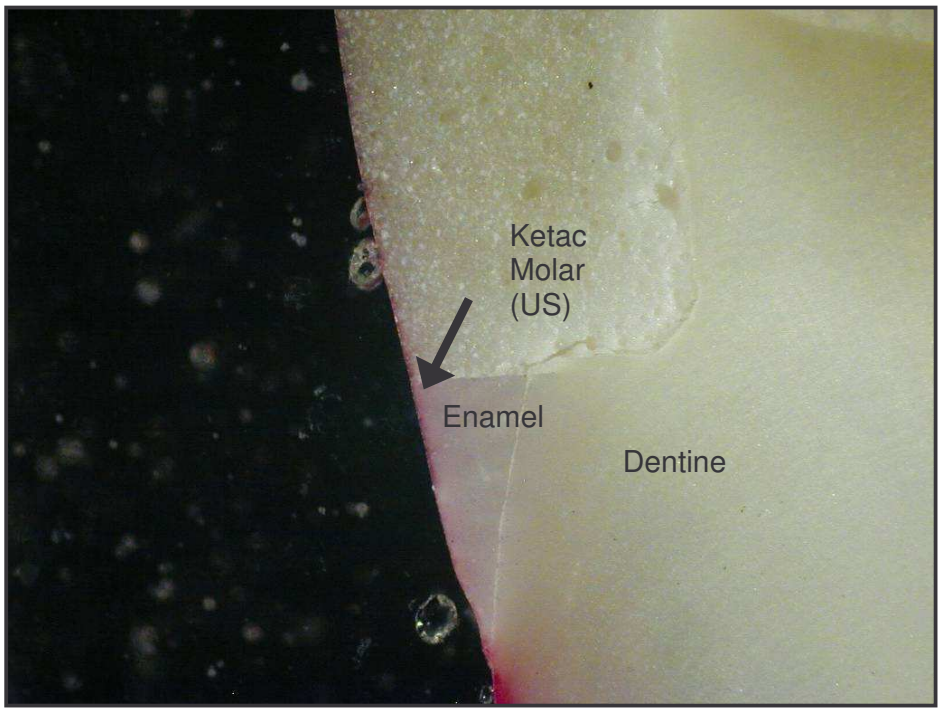

Figure 20: Photomicrograph of a cross section of a tooth restored with Ketac Molar (US) displaying a value of 0 for microleakage of the cervical margin placed in enamel.

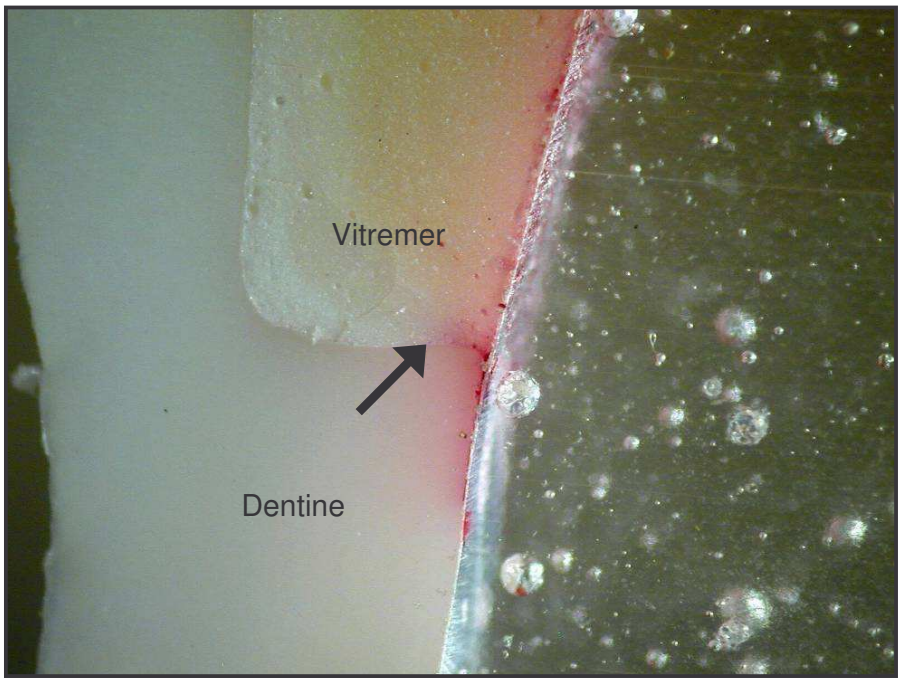

Figure 21: Photomicrograph of a cross section of a tooth restored with Ketac Molar (US) displaying a value of 1 for microleakage of the cervical margin in dentine

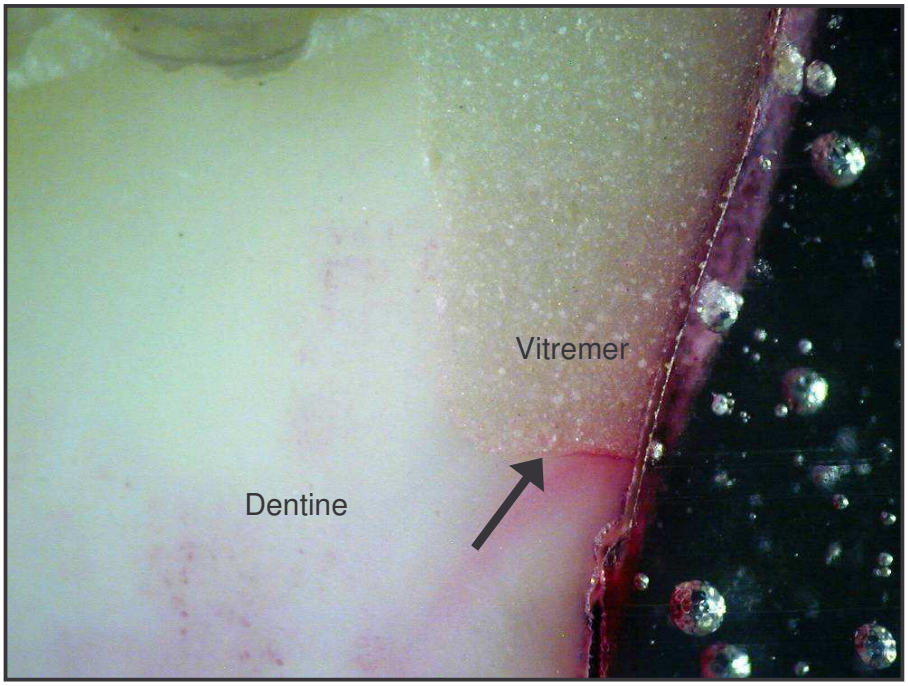

Figure 22: Photomicrograph of a cross section of a tooth restored with Vitremer displaying a value of 2 for microleakage of the cervical margin in dentine

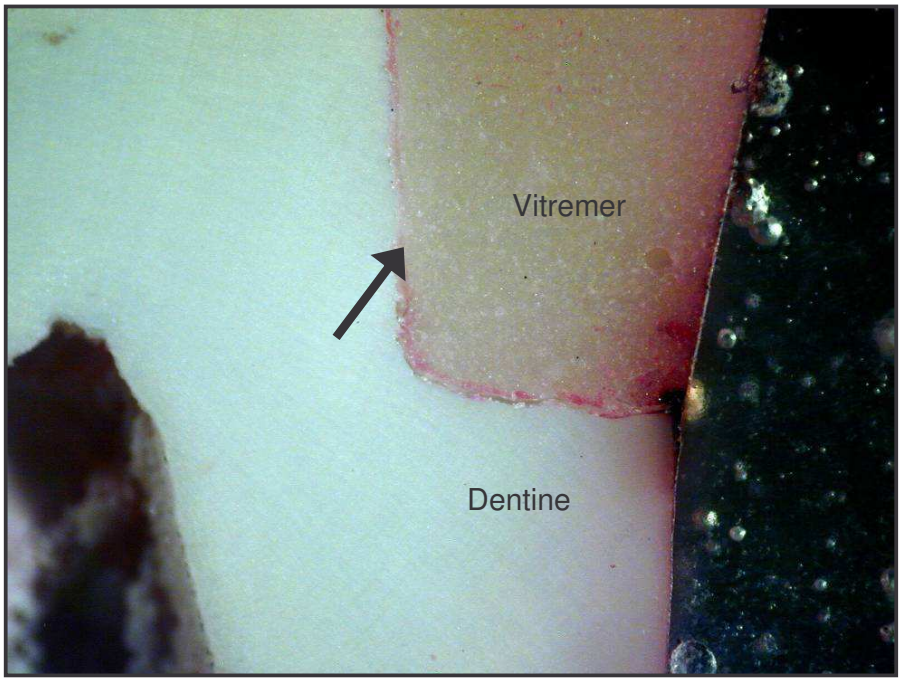

Figure 23: Photomicrograph of a cross section of a tooth restored with Vitremer displaying a value of 3 for microleakage of the cervical margin in dentine

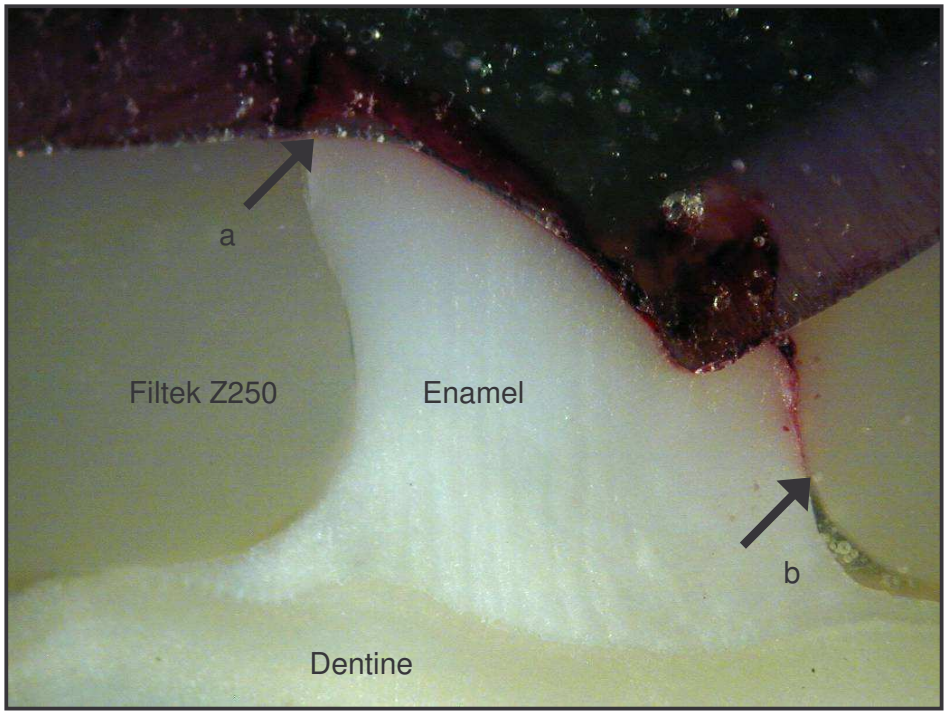

Figure 24: Photomicrograph of a cross section of a tooth restored with Filtek Z250 to display a: microleakage value 0, and b: microleakage value 2

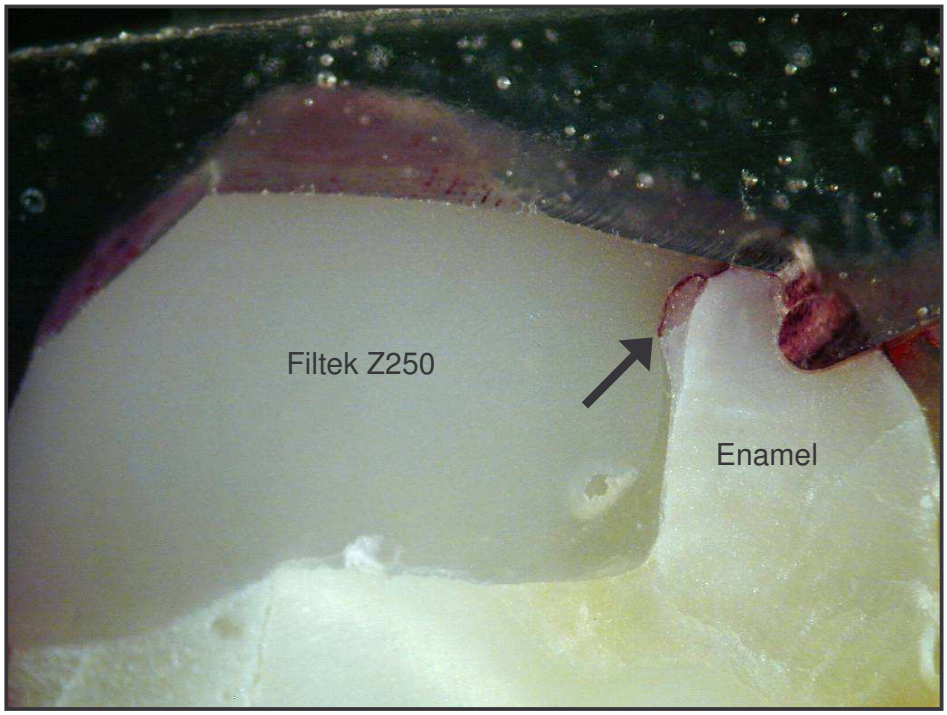

Figure 25: Photomicrograph of a cross section of a tooth restored with Filtek Z250 to display microleakage value 1 at the occlusal cavity margin

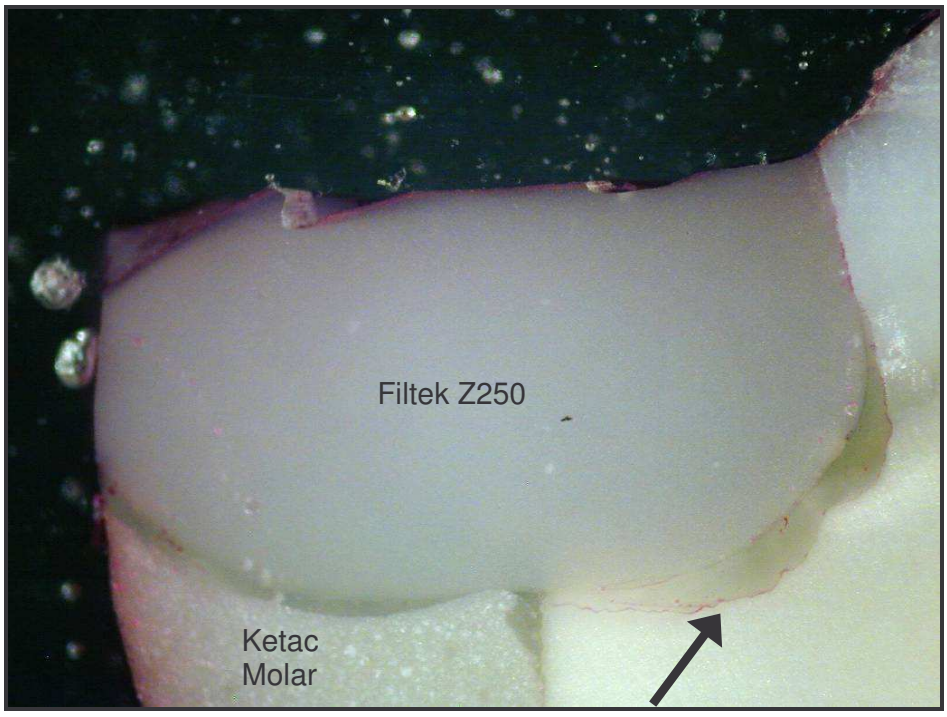

Figure 26: Photomicrograph of a cross section of a tooth restored with Filtek Z250 display microleakage value 3 at the occlusal cavity margin

CHAPTER 3: RESULTS

3 RESULTS

3.1 MEAN MICROLEAKAGE SCORES

Mean microleakage scores were calculated as the sum of microleakage scores given divided by the number of observations made. The number of observations made varied because sections did not always include the relevant cavity margins.

3.2 STATISTICAL ANALYSIS OF MICROLEAKAGE SCORES

The purpose of the study was to find possible differences in leakage at different positions on the teeth between different types of material, taking the thermo-properties of the material and the cervical position of the cavity into account. The factors of the experiment are: *material* (5 levels), *thermocycling* (2 levels: yes/no) and *cervical position* (2 levels: apical/coronal to the CEJ) leading to 20 combinations of the factors, measured at 2 different positions (cervical and occlusal).

Because the raw data did not entirely meet the requirements of normality and homoscedasticity, various data transformations were considered to meet these assumptions. But the final conclusion was that the raw untransformed data would lend itself better to the interpretation of the data, and therefore the raw data was used for the statistical analysis and to compile the graphs.

3.3 RESULTS OF CERVICAL MICROLEAKAGE TESTS

3.3.1 Statistical results of cervical microleakage evaluation

Material	Thermo-cycling	Position	LSMean	Std Dev
Ketac Molar(US)	No	Enamel	0.47	0.54
Ketac Molar(US)	Yes	Enamel	0.71	0.56
Ketac Molar(US)	No	Dentine	1.4	0.54
Ketac Molar(US)	Yes	Dentine	0.85	0.56
Ketac Molar	No	Enamel	1.39	0.65
Ketac Molar	Yes	Enamel	0.47	0.57
Ketac Molar	No	Dentine	1.44	0.90
Ketac Molar	Yes	Dentine	1.77	1.15
Ketac Nano	No	Enamel	0.95	1.12
Ketac Nano	Yes	Enamel	0.64	0.37
Ketac Nano	No	Dentine	1.46	0.56
Ketac Nano	Yes	Dentine	1.51	0.95
Vitremer	No	Enamel	1.4	0.54
Vitremer	Yes	Enamel	0.55	0.43
Vitremer	No	Dentine	2.95	0.15
Vitremer	Yes	Dentine	1.52	0.96
Z250	No	Enamel	0.55	0.42
Z250	Yes	Enamel	0.8	1.01
Z250	No	Dentine	2.65	0.60
Z250	Yes	Dentine	2.82	0.33

Table 1: Mean cervical microleakage scores of individual materials.

Not-thermocycled vs Thermocycled	Enamel	Dentine
Ketac Molar (US)	0.44	0.08
Ketac Molar	0.004	0.29
Ketac Nano	0.32	0.87
Vitremer	0.007	<.0001
Z250	0.42	0.57

Table 2: *p*-values for microleakage comparisons under thermocycling conditions.

Dentine vs enamel cervical margins	Not-thermocycle	Thermocycle
Ketac Molar (US)	0.003	0.67
Ketac Molar	0.87	<.0001
Ketac nano	0.1	0.006
Vitremer	<.0001	0.0022
Z250	<.0001	<.0001

Table 3: *p*-values for microleakage comparisons of different cervical positions.

Not-thermocycled, enamel	1	2	3	4	5
1. Ketac Molar (US)		0.004	0.13	0.003	0.81
2. Ketac Molar	0.004		0.16	0.97	0.008
3. Ketac Nano	0.13	0.16		0.15	0.2
4. Vitremer	0.003	0.97	0.15		0.007
5. Z250	0.81	0.008	0.2	0.007	

Table 4: *p*-values for microleakage comparisons between the different materials placed in enamel and not thermocycled, where: 1 = Ketac Molar (US), 2 = Ketac Molar, 3 = Ketac Nano, 4 = Vitremer, 5 = Z250.

Thermocycled, enamel	1	2	3	4	5
1. Ketac Molar (US)		0.44		0.59	0.79
2. Ketac Molar	0.44		0.59	0.81	0.3
3. Ketac Nano	0.81	0.59		0.77	0.61
4. Vitremer	0.59	0.81	0.77		0.42
5. Z250	0.79	0.3	0.61	0.42	

Table 5: *p*-values for microleakage comparisons between the different materials placed in enamel and thermocycled, where: 1 = Ketac Molar (US), 2 = Ketac Molar, 3 = Ketac Nano, 4 = Vitremer, 5 = Z250.

Not-thermocycled, dentine	1	2	3	4	5
1. Ketac Molar (US)		0.89	0.83	<0.0001	0.0001
2. Ketac Molar	0.89		0.94	<0.001	0.0002
3. Ketac Nano	0.83	0.94		<0.0001	0.0002
4. Vitremer	<0.0001	<0.001	<0.001		0.34
5. Z250	0.0001	0.0002	0.0002	0.34	

Table 6: *p*-values for microleakage comparisons between the different materials placed in dentine and not-thermocyled, where: 1 = Ketac Molar (US), 2 = Ketac Molar, 3 = Ketac Nano, 4 = Vitremer, 5 = Z250.

Thermocycled, dentine	1	2	3	4	5
1. Ketac Molar (US)		0.003	0.03	0.03	<.0001
2. Ketac Molar	0.003		0.41	0.42	0.001
3. Ketac Nano	0.03	0.41		0.97	<.0001
4. Vitremer	0.03	0.42	0.97		<.0001
5. Z250	<.0001	0.001	<.0001	<.0001	

Table 7: *p*-values for microleakage comparisons between different materials placed in dentine and thermocycled, where: 1 = Ketac Molar (US), 2 = Ketac Molar, 3 = Ketac Nano, 4 = Vitremer, 5 = Z250.

3.3.2 Graphical comparison of materials relative to cervical position with and without thermocycling

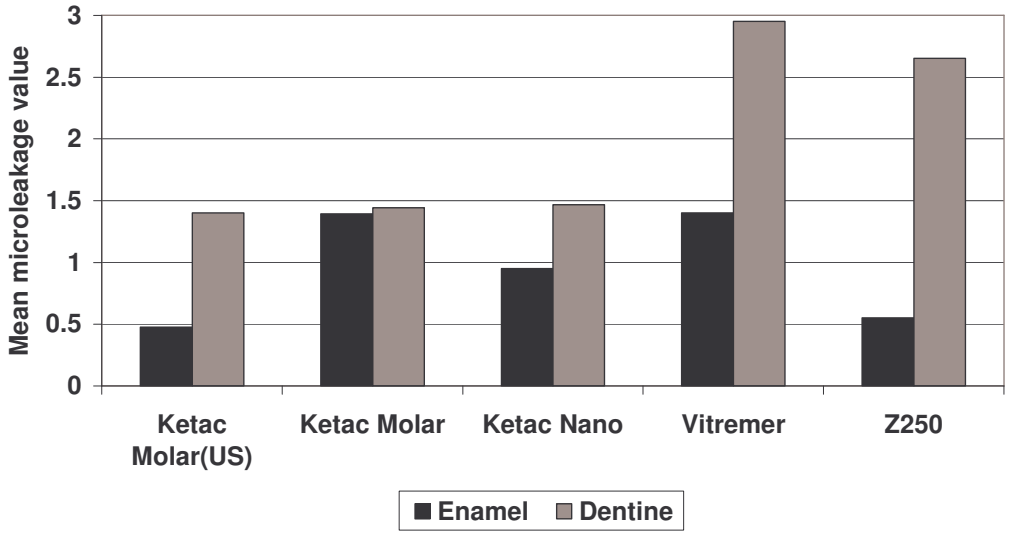

Figure 27: Graphical representation of cervical microleakage of materials placed in enamel and dentine and not thermocycled

3.3.2.1 Results of cervical microleakage of materials placed in enamel and not thermocycled

When cervical cavity margins were positioned in enamel and samples were not thermocycled, three groups could be distinguished. Within the groups there were no statistically significant differences. Ketac Molar (US) and Filtek Z250 was the group that showed the best results, with Ketac Molar (US) performing the best. Next was Ketac N100 which was rated third, but was statistically not significantly different from either the best or the worst group. The groups that performed the worst were Ketac Molar and Vitremer, with Vitremer ranking last (Figure 27).

3.3.2.2 Results of cervical microleakage of materials placed in dentine and not-thermocycled

Two clear groups could be identified when cervical margins were positioned in dentine and samples were not thermocycled. The groups were statistically significantly different from each other, but showed similar results within the groups. The groups that performed the best include Ketac Molar (US), Ketac Molar and Ketac N100 and are ranked here from the least to the most microleakage within that group. Filtek Z250 and Vitremer showed more microleakage in dentine, with Filtek Z250 performing moderately, but not significantly, better than Vitremer (Figure 27).

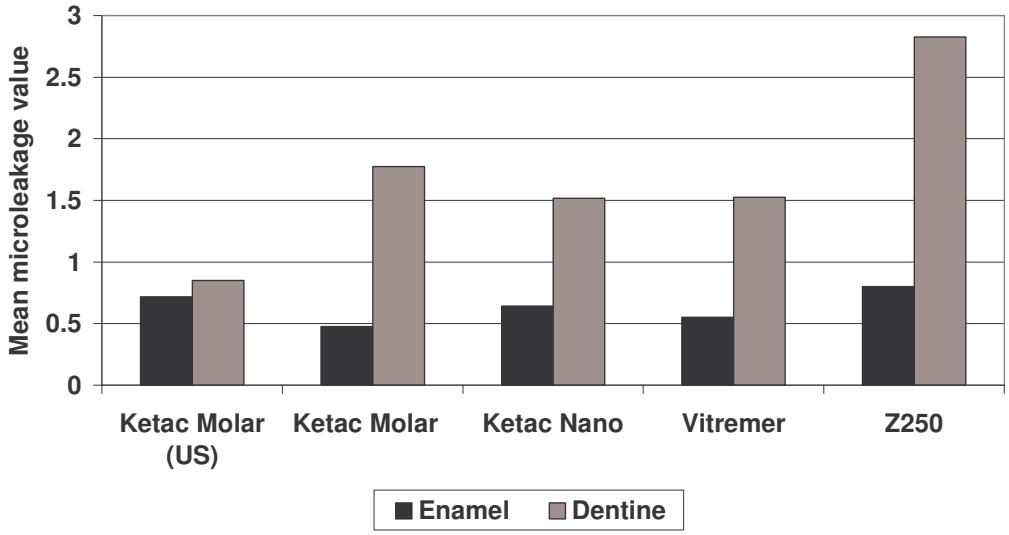

Figure 28: Graphical representation of cervical microleakage of materials placed in enamel and dentine and thermocycled

3.3.2.3 Results of cervical microleakage of materials placed in enamel and thermocycled

Materials that were placed in enamel and thermocycled, performed equally well. Statistically no significant differences were found between the materials, but the materials can be ranked from the least to the most microleakage in the following order: Ketac Molar, Vitremer, Ketac N100, Ketac Molar (US) and Filtek Z250 (Figure 28).

3.3.2.4 Results of cervical microleakage of materials placed in dentine and thermocycled

Thermocycling of samples with cervical margins in dentine further divided the groups of materials. Now three groups could be identified that varied significantly from each other. Ketac Molar (US) stands alone in the top group. Next, showing significantly more microleakage is Ketac N100, Vitremer and

Ketac Molar. The last material is Filtek Z250, demonstrating the most microleakage and significantly more dye penetration than any of the other groups (Figure 28).

3.3.3 The effect of thermocycling and cervical position on the cervical microleakage of individual materials

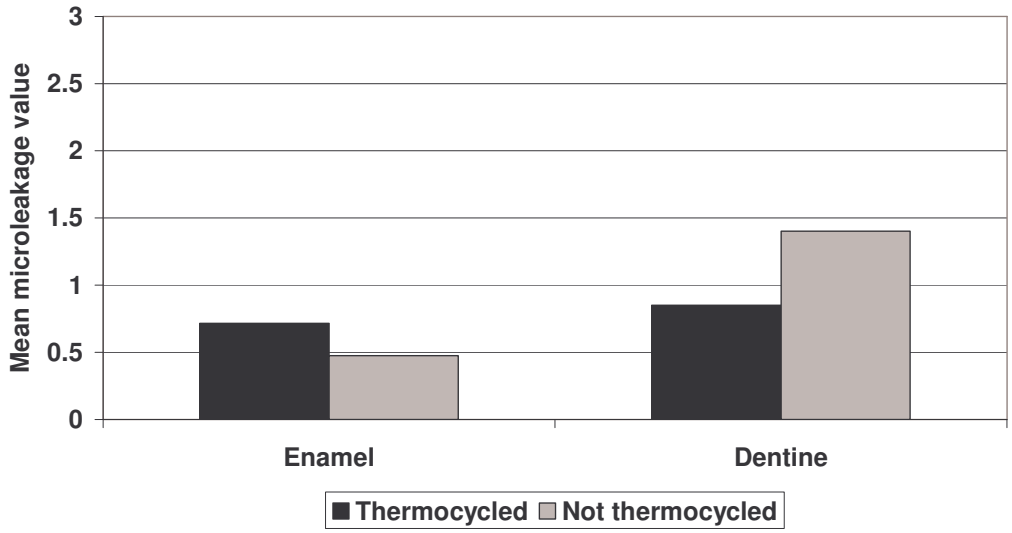

Figure 29: Graphical representation of cervical microleakage of Ketac Molar (US)

For enamel, there is statistically no significant difference between thermocycled and not thermocycled, although the best results were obtained when Ketac Molar (US) was not thermocycled. For dentine, there is statistically no significant difference between thermocycled and not thermocycled, although the best results were obtained when Ketac Molar (US) was thermocycled.

Overall, the best results were obtained for enamel without thermocycling and the worst for dentine without thermocycling (Figure 29).

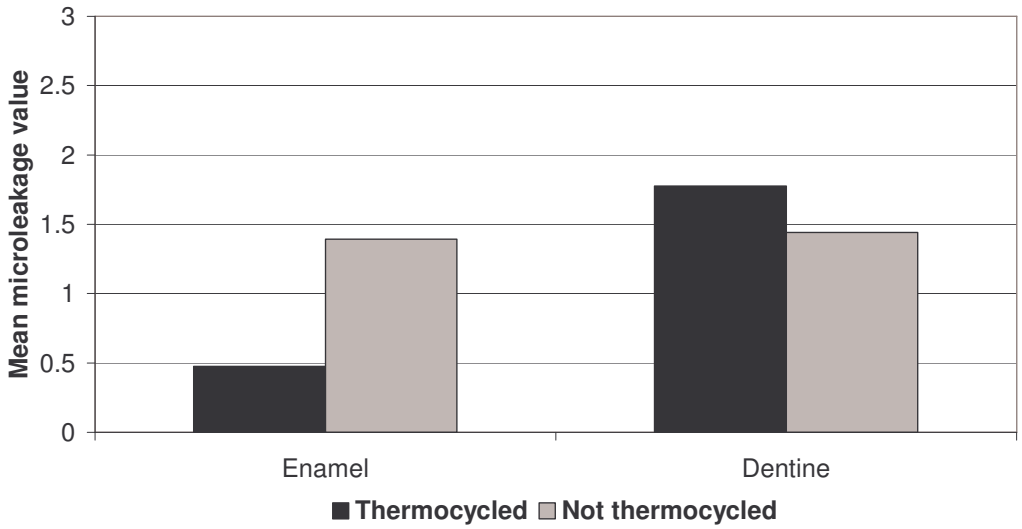

Figure 30: Graphical representation of cervical microleakage of Ketac Molar

For enamel there is a statistically significant improvement when Ketac Molar was thermocycled. For dentine, there is statistically no significant difference between thermocycled and not thermocycled. Overall, the best results were obtained for enamel with thermocycling and the worst for dentine with thermocycling (Figure 30).

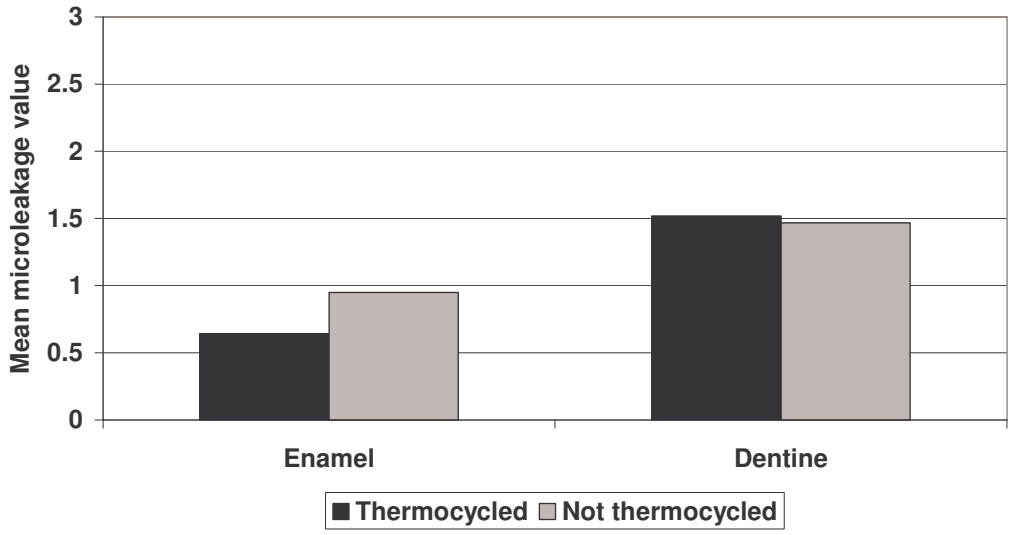

Figure 31: Graphical representation of cervical microleakage of Ketac N100

For enamel and dentine, there is statistically no significant difference between samples that were thermocycled and not thermocycled. Overall the best results for Ketac N100 were obtained for enamel with thermocycling and the worst for dentine with thermocycling (Figure 31).

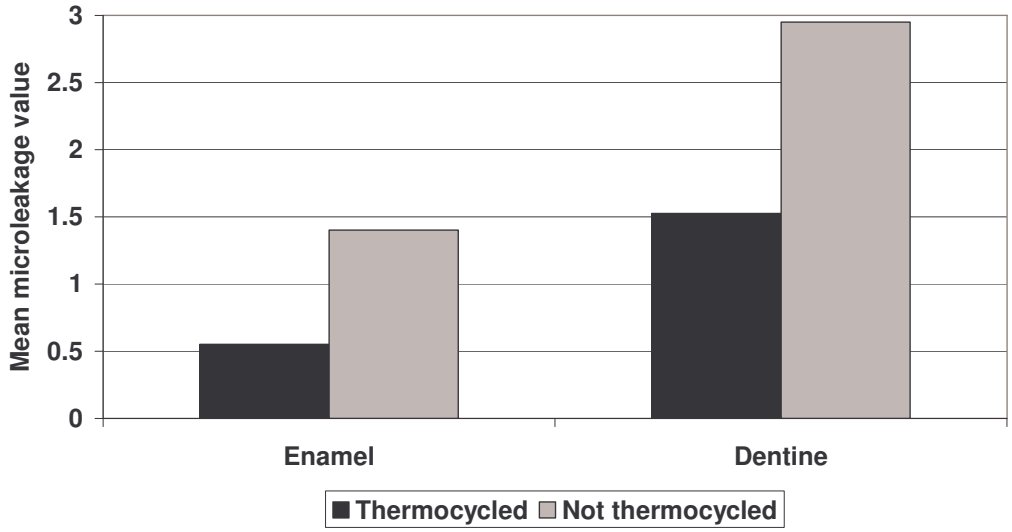

Figure 32: Graphical representation of cervical microleakage of Vitremer

For enamel and dentine, thermocycling significantly decreased the microleakage of Vitremer. Overall the best results were obtained for enamel that was themocycled and the worst for dentine that was not thermocycled (Figure 32).

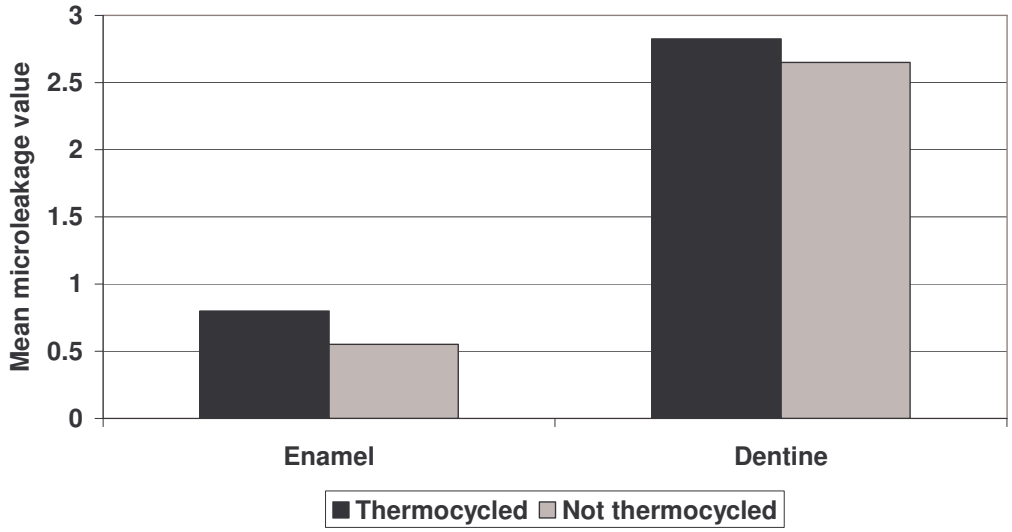

Figure 33: Graphical representation of cervical microleakage of Filtek Z250

For enamel and dentine, there is statistically no significant difference between thermocycled and not thermocycled, although the best results were obtained when Filtek Z250 was not thermocycled. Overall the best results were obtained for enamel without thermocycling and the worst for dentine with thermocycling (Figure 33).

3.4 RESULTS OF OCCLUSAL MICROLEAKAGE TESTS

3.4.1 Statistical results of occlusal microleakage evaluation

Material	Thermocycling	Position	LSMean	Std Dev
Ketac Molar(US)	No	Enamel	0.6	0.52
Ketac Molar(US)	Yes	Enamel	0.39	0.31
Ketac Molar(US)	No	Dentine	0.95	0.69
Ketac Molar(US)	Yes	Dentine	0.37	0.34
Ketac Molar	No	Enamel	1.41	0.98
Ketac Molar	Yes	Enamel	0.12	0.13
Ketac Molar	No	Dentine	0.23	0.42
Ketac Molar	Yes	Dentine	0.43	0.35
Ketac Nano	No	Enamel	0.42	0.54
Ketac Nano	Yes	Enamel	0.55	0.34
Ketac Nano	No	Dentine	0.23	0.9
Ketac Nano	Yes	Dentine	0.35	0.41
Vitremer	No	Enamel	0.7	0.71
Vitremer	Yes	Enamel	0.8	0.44
Vitremer	No	Dentine	0.75	0.57
Vitremer	Yes	Dentine	0.91	0.6
Z250	No	Enamel	0.28	0.25
Z250	Yes	Enamel	0.55	0.26
Z250	No	Dentine	0.4	0.44
Z250	Yes	Dentine	0.55	0.26

Table 8: Mean occlusal microleakage scores of individual materials.

Not-thermocycled vs Thermocycled	Enamel	Dentine
Ketac Molar (US)	0.33	0.008
Ketac Molar	<.0001	0.35
Ketac Nano	0.56	0.58
Vitremer	0.64	0.44
Z250	0.21	0.48

Table 9: *p*-values for occlusal microleakage comparisons under different thermocycling conditions.

Dentine vs enamel cervical margins	Not-thermocycled	Thermocycled
Ketac Molar (US)	0.11	0.93
Ketac Molar	<.0001	0.15
Ketac Nano	0.37	0.35
Vitremer	0.81	0.58
Z250	0.58	1

Table 10: *p*-values for occlusal microleakage comparisons for different cervical positions.

Not-thermocycled, enamel	1	2	3	4	5
1. Ketac Molar (US)		0.0002	0.41	0.64	0.14
2. Ketac Molar	0.0002		<.0001	0.0012	<.0001
3. Ketac Nano	0.42	<.0001		0.21	0.51
4. Vitremer	0.64	0.0012	0.21		0.05
5. Z250	0.14	<.0001	0.51	0.05	

Table 11: *p*-values for occlusal microleakage comparisons between different materials placed in enamel and not-thermocycled, where: 1 = Ketac Molar (US), 2 = Ketac Molar, 3 = Ketac Nano, 4 = Vitremer, 5 = Z250.

Thermocycled, enamel	1	2	3	4	5
1. Ketac Molar (US)		0.22	0.46	0.05	0.46
2. Ketac Molar	0.22		0.05	0.002	0.05
3. Ketac Nano	0.46	0.05		0.24	1
4. Vitremer	0.06	0.002	0.24		0.24
5. Z250	0.46	0.05	1	0.25	

Table 12: *p*-values for occlusal microleakage comparisons between different materials placed in enamel and thermocycled, where: 1 = Ketac Molar (US), 2 = Ketac Molar, 3 = Ketac Nano, 4 = Vitremer, 5 = Z250.

Not-thermocycled, dentine	1	2	3	4	5
1. Ketac Molar (US)		0.001	0.001	0.35	0.01
2. Ketac Molar	0.001		1	0.02	0.44
3. Ketac Nano	0.001	1		0.02	0.44
4. Vitremer	0.35	0.02	0.02		0.11
5. Z250	0.01	0.44	0.44	0.11	

Table 13: *p*-values for occlusal microleakage comparisons between different materials placed in dentine and not-thermocycled, where: 1 = Ketac Molar (US), 2 = Ketac Molar, 3 = Ketac Nano, 4 = Vitremer, 5 = Z250.

Thermocycled, dentine	1	2	3	4	5
1. Ketac Molar (US)		0.78	0.91	0.01	0.42
2. Ketac Molar	0.78		0.69	0.03	0.58
3. Ketac Nano	0.91	0.69		0.009	0.35
4. Vitremer	0.01	0.03	0.009		0.09
5. Z250	0.42	0.59	0.35	0.09	

Table 14: *p*-values for occlusal microleakage comparisons between different materials placed in dentine and thermocycled, where: 1 = Ketac Molar (US), 2 = Ketac Molar, 3 = Ketac Nano, 4 = Vitremer, 5 = Z250.

3.4.2 Comparison of the effect of different materials used in the interproximal box on the occlusal microleakage of Filtek Z250 relative to cervical position and thermocycling

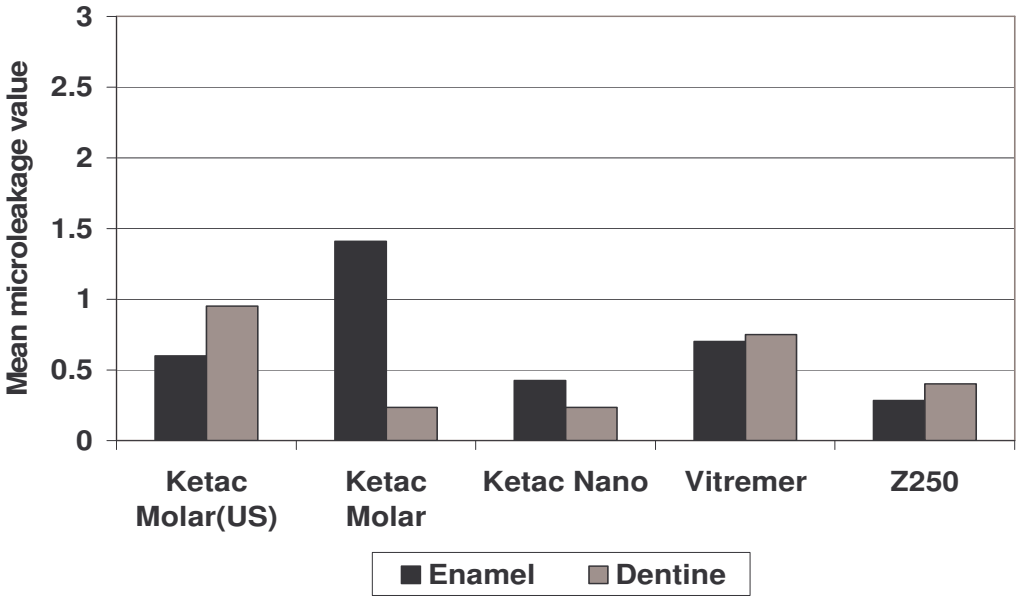

Figure 34: Graphical representation of the occlusal microleakage when cervical margins are placed in enamel and dentine, not thermocycled

3.4.2.1 Results of occlusal microleakage when cervical margin is placed in enamel and not-thermocycled

When cervical margins were placed in enamel and not thermocycled, two groups of occlusal microleakage could be distinguished which were significantly different from each other. The groups that performed the best include Filtek Z250, Ketac N100, Ketac Molar (US) and Vitremer which are listed here from the least to the most microleakage, without statistically significant differences between the groups. The use of Ketac Molar resulted in significantly more occlusal microleakage than any of the other materials used (Figure 34).

3.4.2.2 Results of occlusal microleakage when cervical margin is placed in dentine and not-thermocycled

When the cervical margins where placed in dentine and the samples were not thermocycled, the occlusal microleakage of Filtek Z250 differed in the following way. The use of Ketac Molar, Ketac N100 and Filtek Z250 resulted in the least occlusal microleakage. Although Ketac Molar and Ketac N100 performed better than Vitremer and Ketac Molar (US), Filtek Z250 only showed significantly less microleakage compared to Ketac Molar (US) (Figure 34).

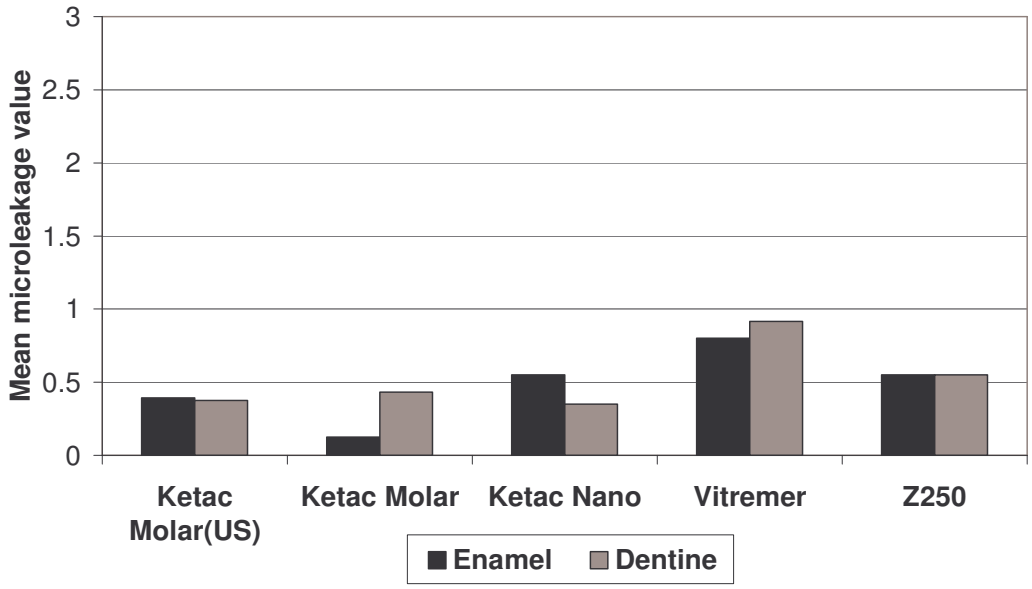

Figure 35: Graphical representation of occlusal microleakage when the cervical margin is placed in enamel and dentine, thermocycled

3.4.2.3 Results of occlusal microleakage when cervical margin is placed in enamel and thermocycled

The only two materials that lead to significant differences in occlusal microleakage when used cervically in enamel and thermocycled are Ketac Molar and Vitremer, with Ketac Molar showing the best results and Vitremer the worst. The rest of the materials did not show significant differences between each other or between Ketac Molar and Vitremer. They are positioned from the least to the most microleakage as follows: Ketac Molar (US), Ketac N100 and Filtek Z250 (Figure 35).

3.4.2.4 Results of occlusal microleakage when cervical margin is placed in dentine and thermocycled

Ketac N100, Ketac Molar (US), Ketac Molar and Filtek Z250 obtained similar microleakage results and are ordered here from the least to the most microleakage. Although the microleakage values of Filtek Z250 is not significantly different from Vitremer, the rest of the materials showed significantly less microleakage than when Vitremer was used to restore the cervical margin (Figure 35).

3.4.3 The effect of thermocycling and cervical position on the occlusal microleakage of Filtek Z250 evaluated for the individual material used cervically

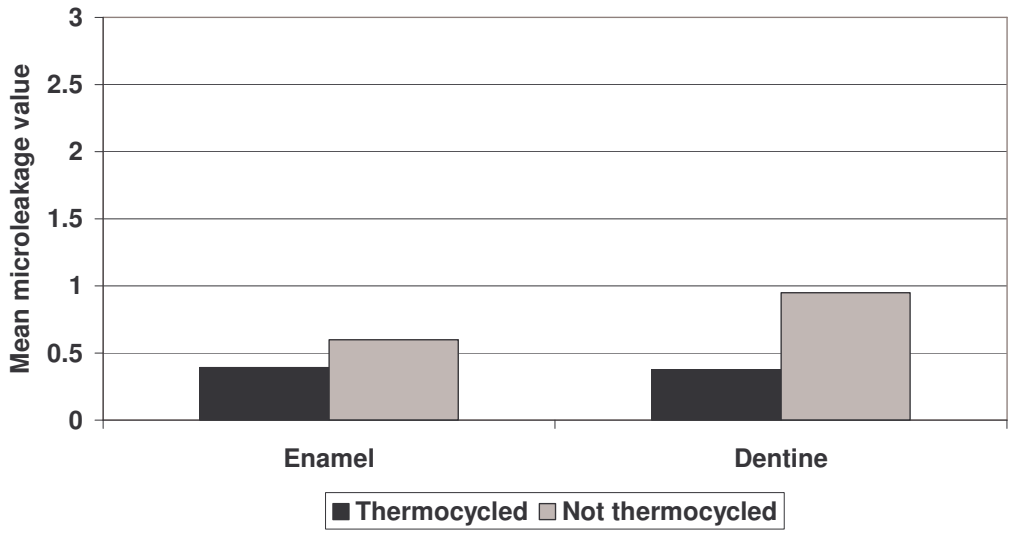

Figure 36: Graphical representation of occlusal microleakage of Filtek Z250 when Ketac Molar (US) is used cervically

Thermocycling reduced the microleakage of the occlusal margin when Ketac Molar (US) was used to restore the cervical cavity margin placed in enamel and reduced it significantly when the cervical cavity margin was in dentine. There was also a significant difference between the occlusal value when the cervical margin was in dentine and not thermocycled and in enamel and thermocycled (Figure 36).

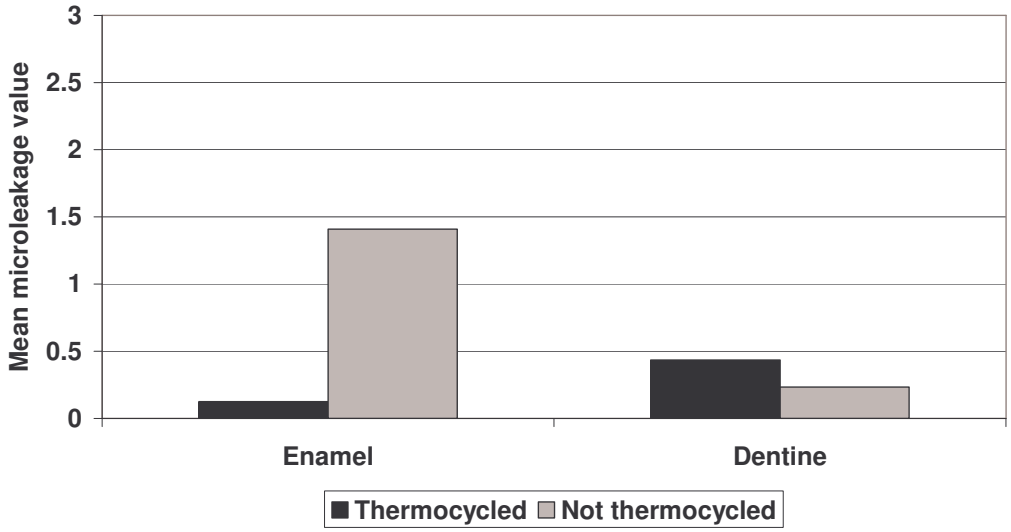

Figure 37: Graphical representation of occlusal microleakage of Filtek Z250 when Ketac Molar is used cervically

When Ketac Molar was used to restore the cervical margin, the only significant difference in microleakage of the occlusal margin could be observed in cavity preparations where the cervical margin was positioned in enamel and the samples were not thermocycled. Thermocycling therefore significantly reduced microleakage of the occlusal margin when the cervical margin was placed in enamel and restored with Ketac Molar (Figure 37).

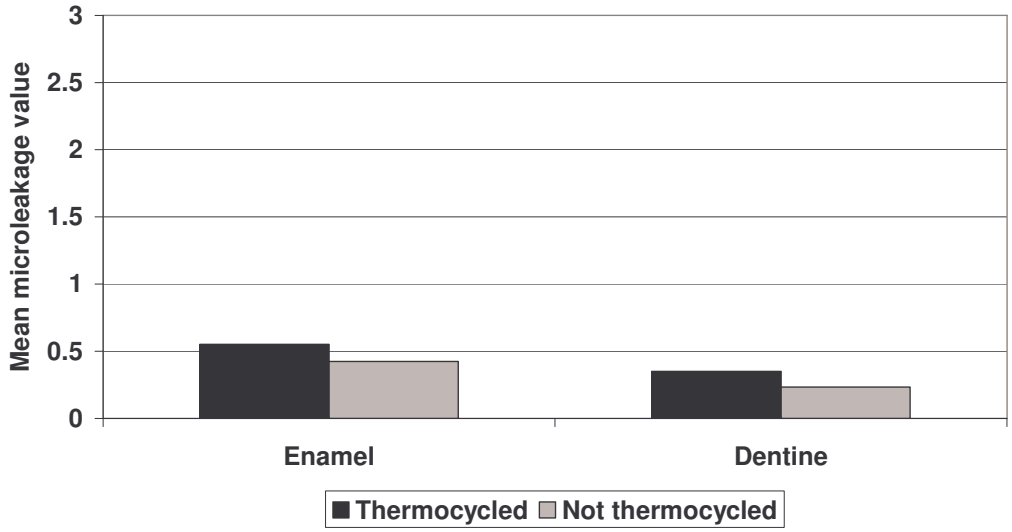

Figure 38: Graphical representation of occlusal microleakage of Filtek Z250 when Ketac N100 is used cervically

No significant differences were observed in the microleakage of the occlusal cavity margins when Ketac N100 was used to restore the cervical margins. Although it can be seen that more microleakage was observed when cervical margins were placed in enamel and when samples were thermocycled (Figure 38).

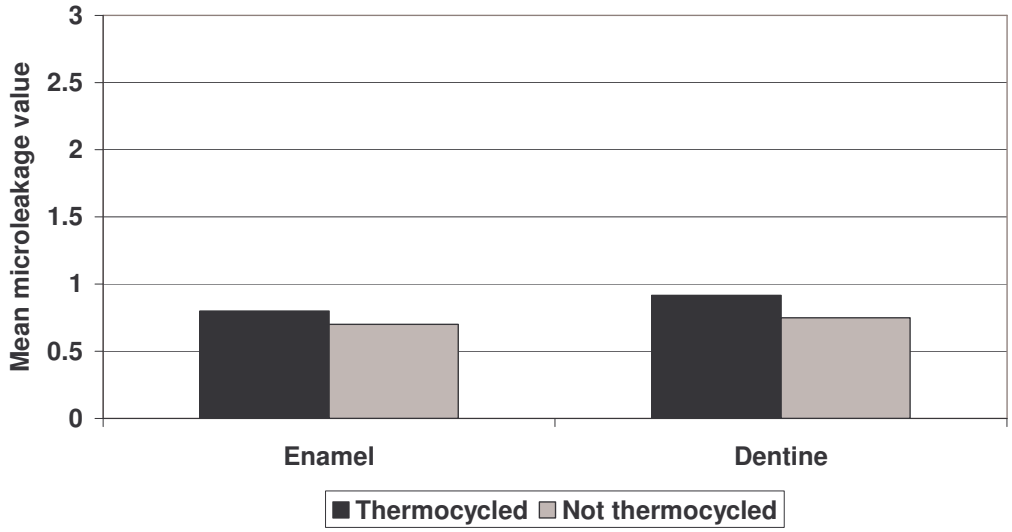

Figure 39: Graphical representation of occlusal microleakage of Filtek Z250 when Vitremer is used cervically

No significant differences were observed in the microleakage of the occlusal cavity margins when Vitremer was used to restore the cervical margins. Thermocycling however appears to cause more microleakage, irrespective of whether cervical margins are placed in enamel or dentine (Figure 39).

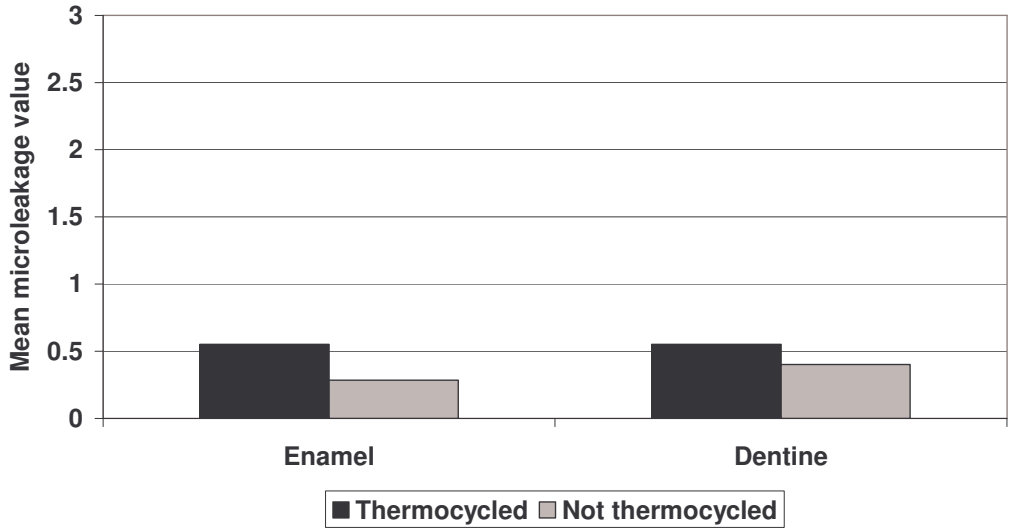

Figure 40: Graphical representation of occlusal microleakage of Filtek Z250 when Filtek Z250 is used cervically

No significant differences were observed in the microleakage of the occlusal cavity margins when Filtek Z250 was used to restore the cervical margins. Although it can be said that samples which were thermocycled showed more microleakage in both the enamel and dentine cervical margin groups (Figure 40).

CHAPTER 4: DISCUSSION

4 MICROLEAKAGE

4.1 CERVICAL MICROLEAKAGE

4.1.1 Effect of thermocycling on microleakage

The decrease in microleakage seen after thermocycling of Vitremer in dentine and enamel could be explained by water sorption of the material as a result of the use of unfilled resins (Attin *et a*l., 1995; Feilzer *et al*., 1995b; McLean 1996; Yap 1996). This water sorption may even reverse the shrinkage stress into a compressive stress (Feilzer *et al*., 1995b). The water sorption also compensates for the difference between the COTE of the RMGI and the tooth structure (Doerr, Hilton and Hermesch, 1996). Materials that are hand mixed, such as Vitremer, also lead to the inclusion of air bubbles that expand in volume during thermocycling (Davidson 1994). The low modulus of elasticity can also allow for some flexibility to compensate for the internal stress of the resin composite during cure (Davidson, 1994).

Thermocycling also reduced the cervical microleakage of Ketac Molar when the cavity margin was placed in enamel. This could possibly be explained by the added opportunity for the conventional GI to complete its prolonged setting reaction (Davidson, 1998), but unfortunately does not explain why this was only true for margins in enamel.

In this study thermocycling of the resin composite resulted in a non-significant increase in microleakage of the resin composite, which was also observed in other studies (Wahab, Shaini and Morgano, 2003; Kubo et al., 2004). Bullard

(1988) and Doerr (1996) attribute this observation to the difference in COTE between tooth and restorative material that will manifest as increasing microleakage with increasing thermocycling and temperature variations.

The literature is undecided about the clinical significance of thermocycling. The effect of thermocycling should vary between different materials because the difference in COTE will manifest as increasing microleakage with increasing thermocycling and temperature variations, and should become more apparent the greater the difference in COTE between the tooth structure and restorative material (Bullard, Leinfelder and Russel, 1988; Doerr, Hilton and Hermesch, 1996). To this effect the shear bond strength of total-etch adhesives decreased after specimens were subjected to thermocycling (El-Araby and Talic, 2007), and microleakage increased (Wahab, Shaini and Morgano, 2003; Kubo *et al.,* 2004).

It is important to select a material that will withstand the effects of thermocycling which simulates the temperature fluctuations of the oral cavity and the natural aging process. The fact that the resin composite showed a moderate increase in microleakage after thermocycling is probably not clinically significant, while the improvement seen with the GI should be weighed against the decrease in microtensile bond strength seen over time (De Munck *et al.,* 2004). But because temperature fluctuations are not the only stress to which a restoration is subjected, the significance of the effect of thermocycling should not be over emphasised.

4.1.2 Effect of position of cervical margin and material selection on microleakage

4.1.2.1 Resin composite

The use of a resin composite (Filtek Z250 with SBMP) consistently resulted in high microleakage values when the cervical margins of the Class II restorations were placed in dentine. This finding is consistent with the current literature (Dietschi et al., 1995; Opdam, Reuters and Burgersdijk, 1998; Aranha and Pimenta, 2004; Kenyon, Frederickson and Hagge 2007). A possible explanation may be that as the enamel approaches the CEJ, it becomes increasingly aprismatic leading to poor bond structure. From as much as 1.5 mm coronal to the CEJ, both bond quality and strength of resin composite to tooth structure are equivalent to the bond achieved to dentine rather than to enamel (Hilton and Ferracane, 1999). Another explanation for the poor performance of Class II composite restorations with margins in dentine is ascribed to the dimensional changes of the composite (Prati et al 1994). The shrinkable bulk of composite can be reduced by using a sandwich material such as a RMGI (Roulet 1994).

Microleakage of cervical cavity margins is often more pronounced than microleakage of occlusal cavity margins. The cervical margin in Class II cavities is sometimes difficult to access by the operator and the close proximity to the gingival margin make moisture control problematic. Increased microleakage in this area has been attributed to the structure of enamel and dentine close to the CEJ, where enamel is often aprismatic and proper etching patterns are not seen (Hilton and Ferracane, 1999). An adequate margin of

enamel protects against microleakage by forming a proper mechanical bond with the bonding intermediate after acid etching (Buonocore, 1955).

Although bonding of composite resin to enamel might be the accepted norm (Loguercio *et al.,* 2004), the general consensus of the literature agrees that bonding to dentine remains problematic (Dietschi *et al.,* 1995; Hilton, Schwartz and Ferracane, 1997). Composite materials still present difficulties when used directly in posterior restorations (Roulet, 1997). Cervical margins are often located in dentine, especially during the replacement of failed restorations (Dietrich *et al.,* 1999).

The perceived difficulties of bonding to dentine relates amongst other to the organic structure of dentine (Burke, Combe and Douglas, 2000). Increased microleakage may result from insufficient penetration of the bonding agent into the demineralised dentine (Thonemann *et al.,* 1999), which could occur because of collagen collapse when dentine is desiccated or by inadequate saturation with resin monomers (Pashley and Carvalho, 1997; Tay, Gwinnett and Wei, 1998). Another reason is the dimensional changes of the composite (Prati *et al.,* 1994; Davidson and Feilzer, 1997) which could be diminished if the shrinkable bulk of composite could be reduced as in the sandwich technique (Roulet, 1994). RMGI are able to reduce microleakage in high C-factor cavity preparations by virtue of their greater elasticity, and by reducing the contraction stress within the composite (Kemp-Scholte and Davidson, 1990).

The results of this study confirm the observation that resin bonding to dentine remains problematic. All of the materials performed poorer and showed more

microleakage in dentine than in enamel. Individual materials further improved on the microleakage of Filtek Z250 in dentine, with Vitremer that was not-thermocycled, as the only exception to the rule.

4.1.2.2 Resin-modified glass-ionomer

The use of RMGI in the open sandwich technique has been shown to improve the marginal seal and adaptation of direct Class II restorations as compared with base or total bond restorations (Friedl et al., 1997; Dietrich et al., 1999; Hagge et al., 2001; Wibowo and Stockton 2001) and is in agreement with the results obtained with Ketac Nano used as a nano-ionomer in this study.

Vitremer, however, only succeeded in reducing microleakage significantly in dentine when samples were also thermocycled, which is in contrast with the results described by other authors (Burgess et al., 1998; Van Dijken et al., 1998; Loguercio et al., 2002) who showed promising results with a combination of Vitremer and Z100.

RMGI is less sensitive to saliva or blood contamination which is likely to occur when the cervical margin extends apical of the CEJ (Momoi *et al.,* 1997, Dietrich *et al*., 2000), and is relatively forgiving if the cervical dentine is accidentally acid etched (Dietrich *et al.,* 2000).

4.1.2.3 Conventional glass-ionomer, and glass-ionomer set with ultrasound

The fact that the conventional GI performed so well is in contrast with the finding of Garcia-Godoy (1988) that the initial bond between GI and tooth structure may be insufficient to withstand the polymerisation contraction stress

of the resin composite. The fact that the ultrasonically cured GI performed so well may, conversely, be ascribed to the fact that the material reached its final characteristics in a far shorter time than the conventionally cured GIs (Towler *et al.,* 2001). However, should the shrinkage stress of the material be sufficient to disrupt the bond between the material and the tooth structure, it is very probable that the tissue will remain protected from the oral environment by a thin film of cement (Davidson, 1998).

Although current literature most often use RMGI in the open sandwich technique, this technique originated (McLean and Wilson, 1977) when a resin composite laminate was used over a conventional GI restoration to reduce the limitations of composite resin restorations, specifically the lack of permanent adhesion to tooth structure (McLean, 1987). This technique however soon became almost obsolete because of reports of a continuous loss of material from the interproximal surface in Class II restorations (Welburry & Murray, 1990; Knibbs, 1992; van Dijken, 1994; Yap, Mok and Pearson, 1997). Therefore, no recent studies report on the sealing ability of conventional GI used in the open sandwich technique in Class II restorations, and deductions are made on the general sealing ability of GI restorations.

Gordon *et al.,* (1985) observed the least microleakage in the dentine margins of Class V cavities, when Ketac-bond GI was used to bond a composite resin, but did not entirely succeed in eliminating microleakage. Doerr, Hilton and Hermesch, (1996) could not find any differences in microleakage between Class V cavities restored with GI or RMGI, and observed slight reductions of microleakage after thermocycling. GI and RMGI liners also succeeded in

reducing microleakage of composite restorations in the study by Sidhu (1992), and the effect was not susceptible to thermocycling.

Ketac Molar was used as conventional GI in the present study, and consistently performed statistically significantly better than the total bond restorations of Filtek Z250. It has been proposed that the conventional GI will deliver poorer results than the RMGI because the material is too weak to withstand the polymerisation stress of the resin composite (Garcia-Godoy, 1988), but in this study statistically no significant differences could be found between the conventional GI and the RMGI. The Ketac Molar samples cured by ultrasound, in fact, performed the best of all the materials in dentine, regardless of thermocycling. This could possibly be attributed to the material reaching its final characteristics in a far shorter time (Towler *et al.,* 2001), and therefore being able to resist the polymerisation contraction stress of the setting resin composite. Should the shrinkage stress of the material be sufficient to disrupt the bond between the material and the tooth structure, it is very probable that the tissue will remain protected from the oral environment by a thin film of cement, while the fracture proceeds cohesively through the cement (Davidson, 1998).

4.2 OCCLUSAL MICROLEAKAGE

The difference between occlusal and cervical microleakage of Class II cavities has often been tested, whether the cervical margin is placed in enamel or dentine. Cervical margins and restorations placed *in vivo* showed more microleakage than occlusal margins and restorations placed *in vitro* (Ferrari and Davidson, 1996).

A direct comparison between cervical and occlusal microleakage could not be made in this study since different materials were used to restore these surfaces. The goal was rather to determine if there was an interaction between the material used cervically and microleakage found occlusally. The rationale was two fold: to reduce the bulk of resin composite, and to provide an elastic base to compensate for polymerisation shrinkage. So far, research has only focused on the ability of the sandwich technique to reduce cervical microleakage, without establishing whether it has any effect on occlusal microleakage.

During the development of the sandwich technique, studies confirmed that a stable bond exists between the sandwich material and the resin composite laminate (Sneed and Looper, 1985; Welburry and Murray, 1990; Knibbs, 1992; Fortin, Vargas and Swift, 1995) which is only limited by the cohesive strength of the sandwich material (Kerby and Knobloch, 1992; Farah, Orton and Collard, 1998). Therefore it was anticipated that an interaction could be possible. The opposite was also possible, that the polymerisation shrinkage stress of the resin could disrupt the cervical bond (Mount, 1994b; Garcia-Godoy, 1998), especially if the cervical bond is slow to form as in the conventional GI (Davidson, 1998). The results of Reid *et al.,* (1994) however disputed this and Andersson-Wenckert (2002) proved this when the application of a separating liner on the RMGI did not improve its marginal adaptation.

If an unfavourable cavity configuration is lined with an elastic layer, the bulk contraction of the restoration can gain some freedom of movement from the

adhesive sides (Kemp-Scholte and Davidson, 1990; Davidson and Feilzer, 1997). GIs have proven to be an effective layer because of its initial rubbery behaviour (Dauvillier *et al.,* 2000) and their intrinsic porosity (Davidson, 1994).

This could not necessarily be proven by this study, because often, the use of Filtek Z250 in the cervical box resulted in some of the lower leakage scores. Some of the sandwich materials used, in fact increased the occlusal microleakage of Filtek Z250. Especially when Ketac Molar was used in the cervical margin in enamel and not thermocycled; and Ketac Molar (US) was used in the cervical margin in dentine and not thermocycled. The reasons for this are difficult to interpret.

Despite the difficulty in predicting clinical effectiveness of materials, adhesives that perform less well in laboratory studies also tend to be less effective in the clinical environment. Therefore, clinical effectiveness of adhesive restorative materials can be predicted by *in vitro* tests (De Munck *et al.,* 2005). But microleakage is not the only predictor of clinical success, and the data of this, or any *in vitro* microleakage study should therefore be cautiously interpreted, especially since it is often an underestimation of *in vivo* microleakage (Ferrari and Davidson, 1996).

CHAPTER 5: CONCLUSIONS

The results confirmed the following hypotheses:

1. The ultrasonically cured glass-ionomer (GI-US) is superior to the conventional glass-ionomer.

- Ketac Molar (US) proved to be statistically significantly better than Ketac Molar for cervical margins placed in enamel and not thermocycled and cervical margins placed in dentine and thermocycled.

2. The nano-filled resin-modified glass-ionomer is superior to the traditional resin-modified glass-ionomer.

- Ketac N100 proved to be statistically significantly better than Vitremer for cervical margins placed in dentine and not thermocycled.

3. Thermocycling of samples will influence the microleakage of restorations significantly.

- Thermocycling statistically significantly influenced cervical microleakage of Ketac Molar placed in enamel and Vitremer placed in enamel and dentine.

- Thermocycling statistically significantly influenced occlusal microleakage of Filtek Z250 when Ketac Molar (US) was placed in dentine and Ketac Molar was placed in enamel.

4. Positioning of the cervical margin apical to the CEJ will result in greater microleakage.

- Statistically significantly more microleakage was found for cervical margins in dentine than in enamel for the following materials when it was not thermocycled: Ketac Molar (US), Vitremer and Filtek Z250.

- Statistically significantly more microleakage was found for cervical margins in dentine than in enamel for the following materials when it was thermocycled: Ketac Molar, Ketac N100, Vitremer and Filtek Z250.

CHAPTER 6: RECOMMENDATIONS

Within the limitations of this research it is recommended that:

1. Conventional GI and RMGI are used as an intermediate layer in the sandwich technique when the cervical margins of Class II restorations are located apical of the CEJ.

2. Resin composites should not be used when the cervical margins of Class II restorations are located apical of the CEJ.

3. Ketac Molar and Vitremer should not be used as an intermediate layer in the sandwich technique when the cervical margins of Class II restorations are located coronal to the CEJ.

4. It is not necessary to use the sandwich technique when the cervical margins of Class II restorations are located coronal to the CEJ.

5. Future research should investigate the performance of the ultrasonically cured GIs and nano-ionomers compared to their conventional alternatives. The studies should aim to investigate the cervical microleakage, marginal adaptation and longevity of these materials in the oral cavity.

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ADDENDUM

ANNOVA TABLES

The GLM Procedure

Class Level Information		
Class	**Levels**	**Values**
Material	5	Ketac Molar Ketac Molar(US) Ketac Nano Vitremer Z250
Thermocycling	2	No Yes
Position2	2	Dentine Enamel

Number of Observations Read	200
Number of Observations Used	200

The GLM Procedure

Dependent Variable: **CERVICAL AVERAGE**

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	19	110.98875	5.8415132	11.81	<.0001
Error	180	89.0444444	0.4946914		
Corrected Total	199	200.0331944			

R-Square	Coeff Var	Root MSE	CERVICALAVG Mean
0.554852	53.38467	0.703343	1.3175

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Materi*Thermo*Positi	4	6.62083333	1.65520833	3.35	0.0114
Material*Thermocycli	4	10.14583333	2.53645833	5.13	0.0006
Material*Position2	4	16.07513889	4.01878472	8.12	<.0001
Thermocycl*Position2	1	0.01388889	0.01388889	0.03	0.8671
Material	4	19.03180556	4.75795139	9.62	<.0001
Thermocycling	1	4.5	4.5	9.1	0.0029
Position2	1	54.60125	54.60125	110.37	<.0001

The GLM Procedure

Dependent Variable: **OCCLUSAL AVERAGE**

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	19	17.58371528	0.9254587	3.98	<.0001
Error	180	41.81458333	0.23230324		
Corrected Total	199	59.39829861			

R-Square	Coeff Var	Root MSE	OCCLUSALAVG Mean
0.296031	87.4337	0.481978	0.55125

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Materi*Thermo*Positi	4	5.32208333	1.33052083	5.73	2E-04
Material*Thermocycli	4	4.7825	1.195625	5.15	6E-04
Material*Position2	4	2.43666667	0.60916667	2.62	0.036
Thermocycl*Position2	1	0.56003472	0.56003472	2.41	0.122
Material	4	3.83319444	0.95829861	4.13	0.003
Thermocycling	1	0.44336806	0.44336806	1.91	0.169
Position2	1	0.20586806	0.20586806	0.89	0.348

LS MEANS: CERVICAL AVERAGE

Least Squares Means for effect Materi*Thermo*Positi							Pr > |t| for H0: LSMean(i)=LSMean(j)					Dependent Variable: CERVICALAVG								
i/j	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**
1		0.87	0.29	0.00	0.89	0.00	0.06	0.02	0.94	0.12	0.81	0.01	<.0001	0.89	0.79	0.01	0.00	0.01	<.0001	0.04
2	0.87		0.22	0.00	0.98	0.00	0.09	0.03	0.81	0.16	0.69	0.02	<.0001	0.98	0.67	0.01	<.0001	0.01	<.0001	0.06
3	0.29	0.22		<.0001	0.23	<.0001	0.00	0.00	0.33	0.01	0.41	0.00	0.00	0.23	0.43	0.00	0.01	0.00	0.00	0.00
4	0.00	0.00	<.0001		0.00	1.00	0.23	0.44	0.00	0.13	0.00	0.60	<.0001	0.00	0.00	0.81	<.0001	0.81	<.0001	0.30
5	0.89	0.98	0.23	0.00		0.00	0.08	0.03	0.83	0.15	0.71	0.02	<.0001	1.00	0.69	0.01	0.00	0.01	<.0001	0.06
6	0.00	0.00	<.0001	1.00	0.00		0.23	0.44	0.00	0.13	0.00	0.60	<.0001	0.00	0.00	0.81	<.0001	0.81	<.0001	0.30
7	0.06	0.09	0.00	0.23	0.08	0.23		0.67	0.05	0.75	0.04	0.51	<.0001	0.08	0.03	0.34	<.0001	0.34	<.0001	0.87
8	0.02	0.03	0.00	0.44	0.03	0.44	0.67		0.02	0.46	0.01	0.81	<.0001	0.03	0.01	0.60	<.0001	0.60	<.0001	0.79
9	0.94	0.81	0.33	0.00	0.83	0.00	0.05	0.02		0.10	0.87	0.01	<.0001	0.83	0.85	0.00	0.00	0.00	<.0001	0.04
10	0.12	0.16	0.01	0.13	0.15	0.13	0.75	0.46	0.10		0.07	0.33	<.0001	0.15	0.07	0.21	<.0001	0.21	<.0001	0.63
11	0.81	0.69	0.41	0.00	0.71	0.00	0.04	0.01	0.87	0.07		0.01	<.0001	0.71	0.98	0.00	0.00	0.00	<.0001	0.02
12	0.01	0.02	0.00	0.60	0.02	0.60	0.51	0.81	0.01	0.33	0.01		<.0001	0.02	0.01	0.77	<.0001	0.77	<.0001	0.62
13	<.0001	<.0001	0.00	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001		<.0001	<.0001	<.0001	0.34	<.0001	0.69	<.0001
14	0.89	0.98	0.23	0.00	1.00	0.00	0.08	0.03	0.83	0.15	0.71	0.02	<.0001		0.69	0.01	0.00	0.01	<.0001	0.06
15	0.79	0.67	0.43	0.00	0.69	0.00	0.03	0.01	0.85	0.07	0.98	0.01	<.0001	0.69		0.00	0.00	0.00	<.0001	0.02
16	0.01	0.01	0.00	0.81	0.01	0.81	0.34	0.60	0.00	0.21	0.00	0.77	<.0001	0.01	0.00		<.0001	1.00	<.0001	0.43
17	0.00	<.0001	0.01	<.0001	0.00	<.0001	<.0001	<.0001	0.00	<.0001	0.00	<.0001	0.34	0.00	0.00	<.0001		<.0001	0.58	<.0001
18	0.01	0.01	0.00	0.81	0.01	0.81	0.34	0.60	0.00	0.21	0.00	0.77	<.0001	0.01	0.00	1.00	<.0001		<.0001	0.43
19	<.0001	<.0001	0.00	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.69	<.0001	<.0001	<.0001	0.58	<.0001		<.0001
20	0.04	0.06	0.00	0.30	0.06	0.30	0.87	0.79	0.04	0.63	0.02	0.62	<.0001	0.06	0.02	0.43	<.0001	0.43	<.0001	

LS MEANS: OCCLUSAL AVERAGE

Least Squares Means for effect Materi*Thermo*Positi								Pr > |t| for H0: LSMean(i)=LSMean(j)						Dependent Variable: OCCLUSALAVG						
i/j	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**
1		<.0001	0.35	0.62	0.00	0.09	0.51	0.46	1.00	0.38	0.59	0.14	0.02	0.03	0.00	0.01	0.44	0.82	0.14	0.14
2	<.0001		<.0001	<.0001	0.03	0.00	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.00	0.00	0.02	0.01	<.0001	<.0001	<.0001	<.0001
3	0.35	<.0001		0.15	0.02	0.44	0.79	0.85	0.35	0.97	0.70	0.59	0.14	0.22	0.03	0.09	0.88	0.49	0.59	0.59
4	0.62	<.0001	0.15		0.00	0.03	0.25	0.22	0.62	0.17	0.30	0.05	0.00	0.01	0.00	0.00	0.20	0.46	0.05	0.05
5	0.00	0.03	0.02	0.00		0.11	0.01	0.01	0.00	0.02	0.01	0.07	0.35	0.25	0.88	0.49	0.01	0.00	0.07	0.07
6	0.09	0.00	0.44	0.03	0.11		0.30	0.34	0.09	0.42	0.25	0.82	0.49	0.64	0.14	0.35	0.35	0.14	0.82	0.82
7	0.51	<.0001	0.79	0.25	0.01	0.30		0.94	0.51	0.82	0.91	0.42	0.08	0.13	0.01	0.05	0.91	0.67	0.42	0.42
8	0.46	<.0001	0.85	0.22	0.01	0.34	0.94		0.46	0.88	0.85	0.46	0.10	0.15	0.02	0.06	0.97	0.62	0.46	0.46
9	1.00	<.0001	0.35	0.62	0.00	0.09	0.51	0.46		0.38	0.59	0.14	0.02	0.03	0.00	0.01	0.44	0.82	0.14	0.14
10	0.38	<.0001	0.97	0.17	0.02	0.42	0.82	0.88	0.38		0.73	0.56	0.13	0.20	0.02	0.08	0.91	0.51	0.56	0.56
11	0.59	<.0001	0.70	0.30	0.01	0.25	0.91	0.85	0.59	0.73		0.35	0.07	0.11	0.01	0.04	0.82	0.76	0.35	0.35
12	0.14	<.0001	0.59	0.05	0.07	0.82	0.42	0.46	0.14	0.56	0.35		0.35	0.49	0.09	0.25	0.49	0.22	1.00	1.00
13	0.02	0.00	0.14	0.00	0.35	0.49	0.08	0.10	0.02	0.13	0.07	0.35		0.82	0.44	0.82	0.11	0.03	0.35	0.35
14	0.03	0.00	0.22	0.01	0.25	0.64	0.13	0.15	0.03	0.20	0.11	0.49	0.82		0.32	0.64	0.17	0.05	0.49	0.49
15	0.00	0.02	0.03	0.00	0.88	0.14	0.01	0.02	0.00	0.02	0.01	0.09	0.44	0.32		0.59	0.02	0.00	0.09	0.09
16	0.01	0.01	0.09	0.00	0.49	0.35	0.05	0.06	0.01	0.08	0.04	0.25	0.82	0.64	0.59		0.07	0.02	0.25	0.25
17	0.44	<.0001	0.88	0.20	0.01	0.35	0.91	0.97	0.44	0.91	0.82	0.49	0.11	0.17	0.02	0.07		0.59	0.49	0.49
18	0.82	<.0001	0.49	0.46	0.00	0.14	0.67	0.62	0.82	0.51	0.76	0.22	0.03	0.05	0.00	0.02	0.59		0.22	0.22
19	0.14	<.0001	0.59	0.05	0.07	0.82	0.42	0.46	0.14	0.56	0.35	1.00	0.35	0.49	0.09	0.25	0.49	0.22		1.00
20	0.14	<.0001	0.59	0.05	0.07	0.82	0.42	0.46	0.14	0.56	0.35	1.00	0.35	0.49	0.09	0.25	0.49	0.22	1.00	