AN *IN VITRO* STUDY OF POST-RESTORATIVE BLEACHING: EFFECT ON MICROLEAKAGE

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Keywords

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Marginal integrity

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SUMMARY

Today composite resin restorative materials have become the number one filling material because of its superior aesthetic outcomes; however, polymerization shrinkage is the most common drawback of composite resins and with increased demand for aesthetic and whiter teeth through bleaching treatments this could result in an increase in the microleakage associated with composite restorations. Several studies have been undertaken investigating the effect of bleaching agents on the microleakage of composite resin restorative materials but still there exists controversy about whether bleaching increases microleakage or not. The question this study proposes to answer is does bleaching treatment effect composite resin restorations as regards microleakage. **Aim and Objectives:** To assess the effect of bleaching on the marginal integrity of Class V composite resin restorations. To determine the effect of a 6% hydrogen peroxide over the counter and a 38% hydrogen peroxide in-office vital bleaching treatment products on the microleakage of Class V composite restorations. **Materials and Methods:** 60 freshly extracted human molars were used in the study. The roots of the teeth were cut and sealed with Vitremer (3M ESPE, USA). Class V cavities were prepared on the facial surfaces. The cavities were restored with Scotchbond Multi-Purpose Plus dentin bonding system (3M ESPE, USA) and Z100 (3M ESPE, USA) composite resin restorative material according to the manufacturer's instructions. The teeth were randomly divided into three groups (n=20). The first group was the control group, the second and third groups were the experimental groups. The control group was stored in distilled water at 37º C. The first experimental group was bleached with 6% hydrogen peroxide twice daily for 7 days simulating the effect of Crest Whitestrips (Procter & Gamble CO., Cincinnati, OH, USA). The second experimental group was bleached with 38%hydrogen peroxide Opalescence Boost tooth whitening system (ULTRADENT, USA) simulating in-office vital bleaching. After the bleaching treatment, the teeth were subjected to thermal cycling for 100 cycles between 5ºC and 55ºC while immersed in a dye. After vigorous rinsing under tap water, the teeth were embedded in methacrylate blocks and sectioned with a water-cooled microtome through the center of the restoration parallel to the long axis of the tooth. Microleakage was evaluated at the enamel and dentin margins of the class V composite resin restorations

using a stereomicroscope at 10X magnification. **Results and Analysis:** Results were analyzed using a Kruskal-Wallis test. The results of the Kruskal-Wallis test showed that there was a statistically significant difference between the three groups for the enamel margins but a no statistically significant difference between the three groups as regards the dentin margins. A Mann-Whitney *U* test was carried out for a pair-wise comparison to determine which group differed from the others at a significance level of $p \leq 0.05$. There was no statistically significant difference between the control group and the first experimental group for the enamel margins ($p > 0.05$). However there was a statistically significant difference between the control group and the second experimental group ($p \leq 0.05$). There was a statistically significant difference between the first and second experimental groups ($p \leq 0.05$). Wilcoxon signed ranks test showed that there was a statistically significant difference between the dentin and enamel margins ($p \leq 0.05$). **Conclusion and Recommendations:** Z 100 composite resin restorations showed significant microleakage when the margins were in enamel in the control group. The leakage was worse at the dentin margins in all three groups. The in-office power bleaching treatment 38% hydrogen peroxide Opalescence Boost tooth whitening system (ULTRADENT, USA) used in this study increased the microleakage at the tooth-restoration interface in enamel margins. The study recommends delaying of composite resin restorations if 38% hydrogen peroxide is going to be used for the bleaching treatment or changing the already placed restorations after the bleaching treatment with 38% hydrogen peroxide.

DECLARATION

I hereby declare that *An In vitro Study of Post-operative Restorative Bleaching: Effect on Microleakage* is my own work, that it has not been submitted before for any degree or examination in any university, and that all the sources I have used or quoted have been indicated and acknowledged by complete references.

Hassan Manni

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Signed:……

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DEDICATION

To my lovely mother and my brother mahjoub for their constant support and sacrifice.

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CHAPTER 1

INTRODUCTION

The demand for whiter teeth has increased among the population with the introduction of the new vital bleaching systems including nightguard bleaching and bleaching strips. In addition it is now rare to find a patient without any composite resin restorations placed in their teeth.

As all composite resins undergo polymerization shrinkage (Labella *et al*, 1999) and with the increasing demand for bleaching agents (Kugel and Ferreira, 2005), the effect of these agents on the marginal integrity of the restorations with a possible resultant increase in leakage needs to be explored.

Some controversy exists regarding the effect of the bleaching agent on restorative materials. Studies conducted by (Crim, 1992 (a), Barkhordar *et al*, 1997, Waite *et al*, 1998, Shinohara *et al*, 2001, White *et al*, 2003, Ulukapi *et al*, 2003, Turkun and Turkun, 2004) indicated that bleaching treatment may adversely affect the marginal integrity and increase the microleakage of the composite resin restorations.

However other studies done by (Crim, 1992 (b), Pioch *et al*, 2002, Klukowska *et al*, 2008, White *et al*, 2008) indicated that bleaching treatment did not affect the marginal integrity. However, evident from the literature and of great significance is the fact that the same authors found conflicting results in different studies as regards the effect of bleaching agents on the marginal integrity of composite resin restorations and the resultant microleakage.

Microleakage is definitely an important issue in modern dentistry, particularly when new versions of adhesive materials are introduced into clinical practice.

Microleakage has been defined as the passage of bacteria, fluids, molecules or ions along the tooth-restoration interface (Kidd, 1976). This leakage may be clinically undetectable, but is a major factor influencing the longevity of dental restorations as it could cause many severe biological effects in the restored tooth including the recurrence of caries, pulp pathology, hypersensitivity and marginal breakdown of the tooth and or the restoration (Hersek, 2002).

The investigation of microleakage is, therefore, important in the assessment of restorative materials and products applied to these restorations as it could impact on the success or failure of a restoration.

A variety of *in-vitro* methods have been introduced in the study of microleakage including compressed air, neutron activation, electrochemical, fluid filtration, bacteria and the use of dyes (Kidd, 1976, Taylor and Lynch, 1992). In addition, various techniques such as scanning electron microscopy, transmission electron microscopy and electron probe microscopic analysis have also been used to image and measure microleakage (Vassiliadis *et al*, 1996).

CHAPTER 2

LITERATURE REVIEW

2.1 – COMPOSITE RESINS

2.1.1 – Definition

A composite by definition in material science refers to a solid formed from two or more distinct phases such as particles in a metal matrix that have been combined to produce properties superior to or intermediate (improved characteristics) to those of the individual constituents (Jandt and Sigusch, 2009, Hervás-García *et al*, 2006, Labella *et al*, 1999).

It is also a term used in dentistry to describe a dental restorative material which is a mixture of submicron glass filler particles and acrylic resin that forms a solid tooth-colored restoration that can be self- or light hardened at mouth temperature and is called a composite restoration (ADA Council on scientific affairs, 2003).

2.1.2 – Composition of composite restorative materials

There are three structural components in a dental resin-based composite restorative material.

- Matrix - a plastic resin material that forms a continuous phase and binds the filler particles (Hervás-García *et al*, 2006).

- Filler - reinforcing particles and/or fibers that are dispersed in the matrix.

- Coupling agent - which is a bonding agent that promotes adhesion between the filler particles and the resin matrix.

Composite restorative materials are complex blends of polymerizable resins mixed with glass powder filler particles. To bond the glass filler particles to the plastic resin matrix, the filler particles are coated with silane, an adhesive coupling molecule. Other additives are also included in the composite formulations to enhance radiographic opacity for better diagnostic identification, to facilitate curing and to adjust viscosity for better handling properties. Color and translucency of dental composites are modified to mimic the color and translucency of teeth, making them the most esthetic direct filling material currently available (Ferracane, 1995).

2.1.2.1 – Resin matrix

Most of the dental composites use a combination of aromatic and/or aliphatic dimethacrylate monomers such as bis-GMA according to Hervás-García *et al* (2006) which is one of the most widely used ingredients. Another currently used agent is urethane dimethacrylate (UDMA), and both of them are higher molecular weight monomers (Hervás-García *et al*, 2006).

These higher viscosity monomers provide good physical and chemical properties, extended lifetime in the oral environment, reduce polymerization shrinkage to some extent and their biocompatibility is acceptable (Geurtsen, 2000).

Triethylene glycol dimethacrylate (TEGDMA) and ethylene glycol dimethacrylate (EDMA) are called diluents and are lower molecular weight monomers. These lower molecular weight monomers are used to dilute the high viscosity resins but they increase the polymerization shrinkage (Braga and Ferracane, 2004).

These monomers are widely used resin matrix ingredients and form highly cross-linked polymer structures in composites (Labella *et al*, 1999).

2.1.2.2 – Filler particles

The filler particles are added to the resin matrix to increase the physical and mechanical properties of the organic matrix, so incorporating as high a percentage as possible of filler is the primary aim (Hervás-García *et al*, 2006).

The filler particles reduce the thermal expansion coefficient and the overall curing shrinkage and provide radio-opacity for radiological monitoring, improve handling characteristics and improve the aesthetic result (Drummond, 2008).

The translucency of a composite restoration must be similar to that of tooth structure. Thus the indices of refraction of the filler particles must be closely matched to that of the resin (Lehtinen *et al*, 2008). For bis-GMA and TEGDMA, the refractive indices are approximately 1.55 and 1.46, respectively, and a mixture of the two components in equal proportions by weight yields a refractive index of approximately 1.50. Most of the glasses and quartz that are used as fillers have a refractive index of approximately 1.50 which is adequate to achieve sufficient translucency as it is very similar to that of dentine or enamel (Willems *et al*, 1991).

The main filler is silicon dioxide which is formed from the burning of silicon compound in the presence of an oxygen and hydrogen atmosphere to form macromolecular chains of silicon dioxide which are the basis of the inorganic filler particles (Emami *et al*, 2005).

In addition boron silicates and lithium aluminum silicates are also commonly used as filler particles (Kula, 1992). In many composites, the quartz is partially replaced by heavy metal particles such as barium, strontium, zinc, aluminum or zirconium, which are radio-opaque, to facilitate curing and to adjust viscosity for better handling characteristics (ADA Council on scientific affairs, 2003).

2.1.2.3 – Coupling agents

The most commonly used coupling agent is gamma-methacryloxypropyl trimethoxysilane, and is responsible for binding the filler particles to the resin (O'Brien, 2002).

Coupling agents by their silanol groups bind to the filler particle by means of a siloxane bond, and the organosilane methacrylate groups form covalent bonds with the resin as illustrated in figure 2.1: (Wolfgang *et al*, 2005, O'Brien, 2002).

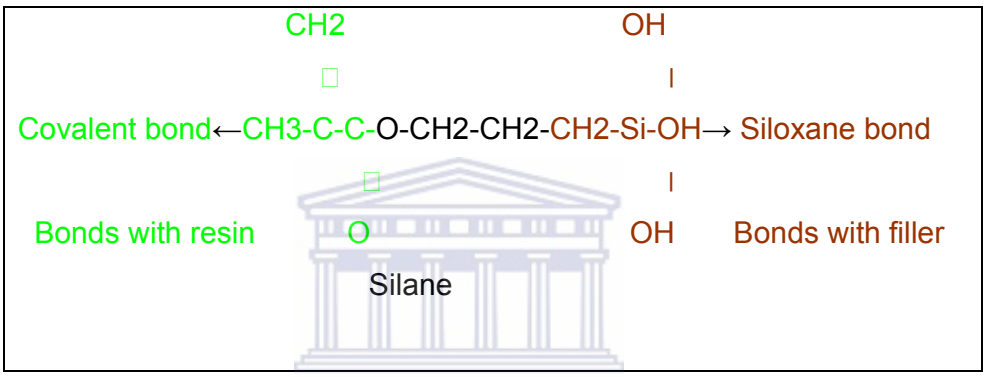

Figure 2.1 Silane Coupling Agents

An inhibitor system such as hydroquinone monomethyl ether is added to maximize the product's storage life prior to curing and its chemical stability thereafter is added to the composite resin. These inhibitors act as absorbers of ultra-violet wavelengths below 350 nm. An example is 2-hydroxy-4-methoxybenzophenone which provides color stability and eliminates the effect of UV light on the amine compounds in the initiator system that causes discolouration in the medium to long term of the composite resin restoration (Hervás-García *et al*, 2006).

2.1.3 – Classification of composite resins

Composite resins may be classified according to initiation of polymerization into chemical, light and dual-curing composite resins (Willems *et al*, 1992). They can also be classified according to filler type as regards the size of the filler particles and indications for use as depicted in table 2.1: (O'Brien, 2002).

Class of composite	**Particle size**	**Clinical use**
Traditional (large particle)	1-50 µm glass	High-stress areas
Hybrids (large particle)	(1) 1-20 µm glass (2) 0.04 µm silica	High-stress areas requiring improved polishability (Classes I, II, IV)
Hybrids (midfiller)	(1) 0.1-10 µm glass (2) 0.04 µm silica	High-stress areas requiring improved polishability (Class IV)
Hybrids (minifilled/SPF)	(1) 0.1-2 µm glass (2) 0.04 µm silica	Moderate stress areas requiring optimal polishability (Class IV)
Packable hybrid	Midfiller/minifiller hybrid, but with lower filler fraction	Situations in which improved condensability is needed (Class I, II)
Flowable hybrid	Midfiller hybrid, but with finer particle size distribution	Situations in which improved flow is needed and/or where access is difficult (Class II)
Homogenous microfill	0.04 µm silica	Low-stress and subgingival areas that require a high luster and polish (Class V and III)
Heterogeneous microfill	(1) 0.04 µm silica (2) Prepolymerized resin particles containing 0.04 µm silica	Low-stress areas and subgingival areas where reduced shrinkage is essential (Class V)

1. SPF, Small –particle filled.

2. Hybrid composites are subdivided according to Young's modulus of elasticity to correspond to that of dentin (Sabbagh *et al*, 2002). Young's modulus of elasticity is a term used to describe the rigidity of a material.

Table 2.1: Classification of Composite Resin (according to Phillip's Science of Dental Materials 2003 citing the work of O'Brien 2002).

2.1.4 – Setting reaction

Dental resins harden when they polymerize. Polymerization is a chemical reaction in which monomers of a low molecular weight are transformed into chains of polymers with a high molecular weight (Ferracane, 1995).

A polymer is a chemical compound consisting of large organic molecules formed by the union of many repeating smaller monomer units. These polymers may also be called co-polymers if they contain two or more different monomer units (Ferracane, 1995).

Composite resins polymerize by an addition polymerization reaction mechanism which is defined as occurring when a reaction between two molecules produces a larger molecule without the elimination of a smaller molecule such as water or alcohol (Daronch *et al*, 2006).

Generally the process of addition polymerization to produce these polymers involves four stages known as: activation, initiation, propagation and termination (Daronch *et al*, 2006).

Addition polymerization of composite resins is initiated by free radicals which are generated by the chemical activation or by external energy activation such as heat or a light source (Geurtsen, 2000).

Based on the activation process dental composite resins may be classified as either chemical or light activated products (Ruyter, 1988).

2.1.4.1 – Chemically activated resins

These are supplied in a two paste system, the one paste contains the benzoyl peroxide (BP) initiator and the other an aromatic tertiary amine activator, which when mixed produce free radicals and an additional polymerization reaction is initiated in the composite resin (Hanks *et al*, 1988) resulting in a set material.

2.1.4.2 – Light activated resins

These used to be activated by ultraviolet light; however prolonged exposure caused retinal damage to the eyes and also heated the oral tissues (Birdsell *et al*, 1977). People who had extreme brightness sensitivity caused by cataract surgery, photosensitizing drugs and so forth had to avoid exposure to the light sources. Prolonged viewing of the light source was detrimental to the staff and had to be avoided. The wearing of protective glasses was mandatory (Birdsell *et al*, 1977).

However light activated resins are now activated by a visible blue-light system. The visible blue light system improves the depth of cure, controls the working time and is also less destructive to the oral and retinal tissues (Hervás-García *et al*, 2006).

The delivery method of light curable dental composites is as a single paste contained in a light-proof syringe. The syringe contains a photosensitizer and an amine initiator. Exposure to visible light in the blue-light region with a wavelength of 400-525 nm produces an excited state of the photosensitizer which then reacts with the amine to form free radicals that initiate the addition polymerization reaction (Stansbury, 2000).

Camphoroquinone (CQ) is a commonly used photosensitizer that absorbs blue light with a wavelength between 400 and 500 nm. Small amounts of CQ, 0.2% by weight or less, in the paste is required for this purpose. The amine initiator, that is suitable for the reaction with CQ, such as dimethylaminethyl

methacrylate (DMAEMA), is also present at low levels, of approximately 0.15% by weight in the composite (Stansbury, 2000).

2.1.5 – Polymerization shrinkage

Several factors are responsible for polymerization shrinkage and include composite type, the shade and translucency of the composite, light intensity and exposure time for the polymerization process. All these factors may negatively affect the integrity of the tooth restoration complex (Giachetti *et al*, 2006).

Polymerization shrinkage is still regarded as the primary negative characteristic of composite resin restorations (Giachetti *et al,* 2006).

Polymerization shrinkage is one of the dental clinician's primary concerns when placing direct resin-based posterior composite restorations. Polymerization of dimethacrylate-based composites is always accompanied by substantial volumetric shrinkage in the range of 2% to 6% (Daniel and Marcos, 2000, Labella *et al,* 1999).

Polymerization shrinkage is one of the most crucial properties of resin based composite restorative materials (Chen *et al*, 2001). It is also considered as one of the major problems that still imposes limitations in the application of direct aesthetic restorative techniques (Loguercio *et al*, 2004, Yazici *et al*, 2004). Composite resins shrink during polymerization mainly because the monomeric units of polymer are located closer to one another than they are in the original monomer state.

The majority of the shrinkage can be resolved before the polymerization gel point by flow that allows composites to change shape thus reducing the contraction stresses. Following gel formation contraction stress build-up occurs since subsequent shrinkage is obstructed because the material is too rigid to allow plastic flow to compensate for the original volume (Chen *et al*, 2001). While restoring an adhesive cavity, the resin composite is restricted from

changing shape except at the free surface because it is bonded to the walls and floor of the rigid tooth structure. This causes further stress development and increases the possibility of microleakage.

During polymerization the conversion of monomer molecules into a polymer network results in a closer packing of the molecules leading to bulk contraction due to the change in density (Venhoven *et al* 1993).

2.1.6 – Factors Responsible for Polymerization Shrinkage Stress

2.1.6.1 – Filler Content

Composite resins consist of a polymer matrix and a filler material. Shrinkage is a direct function of the volume fraction of polymer matrix in the composite resin. The more monomer entities that unite into polymer chains to form networks, the higher the composite contraction (Hervás-García *et al*, 2006).

On the other hand, the space occupied by the filler particles does not participate in the curing contraction. Therefore, the presence of high filler levels is fundamental to reduce shrinkage of the composite during polymerization (Condon and Ferracane, 2000, Giachetti *et al*, 2006).

2.1.6.2 – Degree of Conversion

The degree of conversion is a measure of the percentage of carbon-carbon double bonds that have been converted to single bonds to form the polymeric resin. The higher the degree of conversion, the better the strength and wear resistance of the composite resin (Venhoven *et al,* 1993, Braga and Ferracane, 2002). It has also been found that there is a direct relationship between the degree of conversion and volumetric shrinkage of the composite resin (Venhoven *et al,* 1993).

2.1.6.3 – Elastic Modulus

An *in-vitro* study has shown that the interfacial stress during the setting shrinkage of a resin composite is positively correlated with the stiffness rate of the setting material known as the elastic modulus or Young's modulus (Feilzer *et al,* 1990). Therefore, at a given shrinkage value, the most rigid material (the material showing the highest elastic modulus) will cause the highest stress. The elastic modulus also increases as the polymerization reaction proceeds (Feilzer *et al,* 1990).

The higher the elastic modulus and polymerization shrinkage of the composite, the higher the contraction stresses. Stress is determined by the volumetric shrinkage multiplied by the elastic modulus according to Hooke's Law (Braga *et al*, 2005).

The modulus of elasticity of enamel (33.6 GPa) and dentine (11.7 GPa) is greater than that of composites at 10.5 GPa for condensable composites (Agosta and Estafan, 2003). Micromovement of resin may occur under stress because composite resin is a flexible material with elastic properties due to the internal weak bonds, while enamel does not deform under compressive strength before fracturing. This may cause bond failure at the tooth restoration interface resulting in microleakage and percolation of fluids or a fracture of the tooth surface (Agosta and Estafan, 2003).

2.1.6.4 – Water Sorption

The phenomenon of water sorption of resin composites and their resulting hygroscopic expansion could compensate for the resin composite shrinkage. As a result, hygroscopic expansion will contribute to the relaxation of shear stress parallel to the adhesive interface. In contrast to the rather rapid polymerization contraction resulting in stress development the hygroscopic expansion providing relief proceeds slowly and may require days. Neither the original contraction stress nor the hygroscopic expansion will be uniform throughout the restoration because water sorption is a gradual process taking many months to

complete. Although reducing polymerization shrinkage stress, water sorption causes an alteration of color stability because if the composite can absorb water, it can also absorb other fluids leading to discoloration of the composite resin restoration (Feilzer *et al,* 1990, Feilzer *et al,* 1988).

In addition water sorption also has a negative effect on the mechanical properties of the composite resin as the filler particles can dissolve in water thereby compromising the strength of the composite resin restoration (Retief, 1994).

2.1.6.5 – Cavity configuration factor (C-factor)

There is a relationship between cavity configuration and stress development (Feilzer *et al*, 1987). Flat surfaces and shallow cavities represent the most favorable conditions for the formation of a durable composite-dentin bond. In these cavities polymerization contraction is restricted to one direction only (Giachetti *et al*, 2006).

Accordingly Feilzer *et al*. (1987) developed the C-factor concept which is the ratio of the bonded surfaces to the unbonded surfaces and this has been used to calculate the possible influence of the shrinkage stress. They performed polymerization stress development experiments on cylindrically shaped specimens explaining the concept of the configuration factor.

Box-like class I cavities have five bonded surfaces and only one un-bonded surface of the composite restoration. The C-factor is therefore 5 (5/1) if all of the walls have the same surface area. Class V wedge-shaped lesions have a lower C-factor, usually between 1.5 and 3, depending on the design of the cavities. Therefore most clinical class V restorations have a C-factor value of approximately 1 to 2 implying 1 to 2 bonded surfaces against 1 un-bonded surface and due to their shallow nature may have the same ratios. Values of C-factor close to 1 apply to class IV restorations and composite layers applied to flat or shallowly curved surfaces because it refers to 1 bonded surface against a single unbonded surface, The larger the C-factor, the higher the competition

between the strength of the bond and the forces of polymerization shrinkage (Feilzer *et al*, 1987).

In a large cavity there will be more shrinkage due to the higher volume of the composite resin restorative material used, which, in turn, will cause a higher resultant stress on the cavity walls. This is why a layering technique should always be used when restoring any cavity type, especially deep cavities with a large C-factor (Giachetti *et al*, 2006).

2.1.6.6 – Light-curing and Self-curing Composites

Krejci and Lutz (1991) found self-curing composites to have better marginal adaptation and less microleakage than light –curing composites. The two types of composite resin restorative materials develop different polymerization shrinkage stresses due to two intrinsic factors namely: velocity of polymerization and porosity (Kinomoto *et al*, 1999, Krejci and Lutz, 1991).

The velocity of the polymerization reaction of the light-curing composites is much faster than the self-curing composites as it is a command- set as opposed to a chemical reaction. A lower velocity also results in better adaptation of the restoration to the cavity walls. In addition the velocity of polymerization reaction may also affect the flow capacity of the resin composite (Kinomoto *et al*, 1999).

Porosity, which is usually present in self-curing composites, is a result of incorporating air during the mixing procedure and has been shown to decrease shrinkage stress development. This may be due to the inhibiting effect of the oxygen in the voids on the setting reaction during polymerization (Alster *et al*, 1992). However this then implies that the properties of the composite resin may be compromised as it may not be fully polymerized.

2.1.6.7 – Placement Technique

Applying the composite resin in layers instead of using a bulk technique is suggested to reduce shrinkage stress (Figueiredo *et al*, 2003). The composite is applied in an oblique layer pattern starting form one corner in the base of the cavity to minimize the contraction shrinkage with regards to minimizing the C-factor.

As a result three main factors can be identified to reduce shrinkage stress namely: the use of a small volume of material, a lower cavity configuration C factor, and contact with a minimum number of opposing cavity walls during polymerization of the material (Loguercio *et al*, 2004).

A key factor in the clinical durability of composite restorations is successful attachment of the composite material to the tooth surface. Attachment to the tooth involves effective bonding of an adhesive to two distinctly different substrates, i.e. the highly mineralized enamel and the wet, collagen-rich dentin. Dentin bonding is the process of bonding a resin to conditioned dentin. A dentin bonding agent is a thin layer of resin between conditioned dentin and the resin matrix of a composite resin (Chan *et al*, 1985).

2.2 – MICROLEAKAGE

2.2.1 – Definition

Microleakage is the flow of oral fluid and bacteria into the microscopic gap between a prepared tooth surface and a restorative material (Kidd, 1976, Raskin *et al*, 2003, Matharu 2001).

2.2.2 – Introduction

If the resin material does not penetrate the collagenous network or debonds from it as the resin shrinks during polymerization, a gap will form between the resin and the dentin. This shrinkage may also occur with enamel. Although this

gap is only a few microns wide, it is wide enough to permit bacteria and oral fluids to percolate from the pulp outward or from the oral cavity inward. This leakage has traditionally been termed microleakage (Matharu 2001).

The biocompatibility of a restoration is altered by the leakage process, which may cause a number of undesirable events (Chan *et al*, 1985, Bishop and Briggs, 1995, Mount and Hume, 2005). It may allow bacteria or bacterial products to reach the pulp and cause infection (Bishop and Briggs, 1995, Mount and Hume, 2005).

It may encourage the breakdown of the material, which may result in exposing the body to by-products of the composite material. Breakdown of the composite material increases the gap, thereby promoting more leakage (Matharu 2001).

The restorative materials constantly undergo changes of a thermal nature when placed in the oral environment, due to an intake of food and fluids at varying temperatures (Sidhu *et al*, 2004).

The leakage may also discolor the margins of the restoration, making the tooth-restoration complex aesthetically unacceptable (Hilton, 2002).

2.2.3 – Leakage at micron level

It can be inferred from the above microleakage definition that marginal gaps around a restoration permit bacteria to pass into the tooth-restoration interface (Bishop and Briggs, 1995).

This is considered to be bacterial microleakage, which occurs at a micron level. Numerous studies have shown that once cariogenic bacteria gain entrance to the tooth-restoration interface they are able to proliferate along and within this area with the potential to cause an adverse response from the pulp and recurrent caries (Bishop and Briggs, 1995, Mount and Hume, 2005).

2.2.4 – Leakage at submicron level

It can also be interpreted from the above definition that restorations with marginal gaps that permit ions and molecules to gain access can result in microleakage at a nano level (Matharu 2001). Apparently leakage can occur at the tooth-restoration interface but bacteria may not be able to enter (Matharu 2001).

It is agreed that fluid flow containing ions and molecules permit access with ease into dentinal tubules especially when the dentin surface has been treated with acid-etch or other conditioning agents which result in the removal of dentine plugs and the subsequent opening of the tubules (Mount and Hume, 2005).

2.2.5 – Development of microleakage

There are many factors that can cause microleakage. Polymerization shrinkage of adhesive restorations has been commonly documented where the hardening phase causes a considerable contraction in volume, creating stresses and forming gaps between the cavity walls and the restoration (Rees and Jacobsen, 1989).

Secondly, some restorative materials such as Glass Ionomer Cements have the property of thermal expansion and water absorption, which can result in leakage (Retief, 1994).

Thirdly, long term mechanical loading and thermal changes can cause elastic deformation and physical alteration of both tooth substance and restoration, resulting in microleakage (Hilton, 2002).

2.2.6 – Adverse effects of microleakage

Restorative marginal gaps that permit the ingress of oral fluid are considered a major reason of pulpal reaction and in time pulpal injuries and ultimately pulpal

necrosis (Mount and Hume, 2005). However, it is reported that the most substantial biological effect of microleakage on a restored tooth may be the development of recurrent caries, which accounts for approximately 50% of the causes of clinical failure of restorations (Trowbridge, 1987).

Recurrent caries sometimes referred to as secondary caries can be clinically and radiographically identified at the restoration margins, most frequently on the gingival margins of class II and class V restorations. Recurrent caries may develop from another primary lesion in the vicinity of the restoration or may be initiated at the restoration margin, where dental plaque accumulation followed by demineralization of the tooth is accelerated by the presence of microleakage (Trowbridge, 1987).

2.2.7 – Measurement of microleakage

Microleakage can be demonstrated through techniques which include the use of bacteria, compressed air, chemical and radioactive tracers, electrochemical investigations, scanning electron microscopy and, perhaps most commonly of all, the use of dye penetration studies (Hilton, 2002).

Dye leakage studies are amongst the most frequently used methods for detecting microleakage (Déjou *et al*, 1996). The other methods include the use of color producing micro-organisms, radioactive isotopes including ^{45}**Ca**, ^{131}**I**, ^{35}**S**, ^{22}**Na**, air pressure method, neutron activation analysis, electrochemical studies, scanning electron microscopy, thermal and mechanical cycling and chemical tracers (Taylor and Lynch, 1992).

Most of the studies assessing microleakage are based on only one section. Raskin *et al*, (2003) in a literature review on microleakage of 144 published articles showed that 47% of the researchers used only one section, 20% used two sections, and 12% used three sections and concluded that using only one section did not give an accurate measurement of microleakage because dye penetration varied from one zone to another within the restoration-tooth interface (Tay *et al*, 1995 and Hilton *et al*, 1997) and therefore recommended

that at least three sections be used to avoid under-estimation of the microleakage (Raskin *et al*, 2003). Based on the work of Raskin *et al*, this study will use three sections of each restoration to evaluate microleakage at the tooth-restoration interface.

The limitation of the longitudinal sections was that only the sectioned part of the restored cavity could be examined. The observed section may not necessarily be the best representative of the total leakage distribution (Youngson *et al*, 1998) since dye penetration may vary from one zone to another in the same tooth-restoration interface (Hilton *et al*, 1997). Gale *et al* (1994) reported that microleakage was a three-dimensional phenomenon and that different locations and angles of sectioning might result in completely different dye penetration scores in the interface. This could make it possible for the observers to miss greater dye penetration which could be on the part of the restored cavity that was not exposed, i.e. not in the line of sectioning (Federlin *et al*, 2002).

2.2.8 – Factors influencing microleakage studies

2.2.8.1 – Substrate for microleakage studies

It is well documented that a myriad amount of microleakage research has been done on extracted human teeth although bovine teeth have also been used at times (Hilton, 2002). It was also cited that teeth in living humans are the best substrate for bonding tests and also to conduct microleakage tests due to intrapulpal pressure amongst other factors. However, it is extremely hard to have these studies done *in vivo*; leading to exclusive use of extracted human teeth for *in vitro* studies (Rueggeberg, 1991).

2.2.8.2 – Storage factors

The factors such as time, media and temperature for the storage of extracted teeth and specimens can play a role in microleakage studies. These factors could be related to the period of time after extraction, time before specimen preparation and time after specimen fabrication. In addition, due to the concern

of infective diseases, most extracted teeth are placed in sterilizing/disinfecting solutions for a period of time before changing to another media for storage and this could affect the bonding and the subsequent microleakage in the study.

Research comparing the effect of autoclave and ethylene oxide sterilization procedures on bonding strength with those of non-sterilized specimens, found that there was no difference in shear bond strength and dentin permeability, and that either method of storage could be applied without adversely affecting the study (Pashley *et al*, 1993).

The time factor after extraction has not been specified by most studies. The most common words “freshly extracted” were used to describe sample collection but it seems hard to extrapolate the exact time period from studies that used the “freshly extracted teeth”. Generally, it ranged from minutes to years (Hilton, 2002). An extensive review done by Rueggeberg (1991) concluded that time after extraction has no impact on bond test results. He also concluded that storage time after cavity preparation but before material placement could be more important, and that restorations should be completed immediately after cavity preparation to better simulate the clinical procedures (Rueggeberg, 1991).

Another time factor is storage duration after specimen fabrication. It was reported that there was a remarkable reduction in shear bond strength values and increased gap formation at the cavity floor between 24 hours and six months when the teeth were stored for two months in 70% ethanol, but no marginal gaps were found in the study done with class V restorations evaluating microleakage for two bonding agents with composite resin (Gwinnett and Ju, 1995).

A broad range of medium solutions have been used for the storage of extracted teeth, including formalin, thymol, chloramines, sodium azide, saline and water. These media may have different effects on enamel and dentin. It was found that physiological saline can make enamel softer while distilled water less so and sodium chloride had no effect on enamel surface hardness. It was also found

that formaldehyde is not an appropriate medium for storing extracted teeth as an oxidation process can form formic acid, which causes changes in pH of the medium solution (Rueggeberg, 1991). Therefore influence bonding is influenced by the storage media used for the specimens. The storage media affects or results in severe change in the structure or composition of the enamel or the dentin which is intimately involved in the bonding process.

It seems that dentin was more affected by storage solution than enamel. Teeth stored in saline demonstrated the greatest changes in dentin permeability over time. It was found that the shear bond strength of composite and dentin fluctuate with storage media and time after extraction. It was also reported that ethanol and formalin provided stable results, while the saline results were dramatically variable. The authors also found that microleakage markedly increased in teeth stored in chloramine solution after 28 days, but no further increase was noted for up to 135 days. These changes could be caused by the modification in the dentin due to ion exchanges, changes in collagen framework and dentin tubule structure and composition (Goodis and Allart, 1993).

2.2.8.3 – Cavity design

Cavity design including size, shape and location can be important in microleakage studies because these variables closely relate to bonding efficiency of adhesive materials and could thus result in microleakage (Gale and Darvell, 1999, Hilton and Ferracane, 1999, Hilton, 2002). It has been suggested that it is necessary for cavities to be as standardized as possible so as to eliminate variation among specimens.

Cavity size is an important variable for the microleakage testing of adhesive materials as polymerization shrinkage can be significantly altered by the volume of the restoration. It was reported that the volumetric contraction during the setting phase of resins ranged from 1.0-3.6% by volume after 30 seconds and these shrinkage values can reach a range of 2.8-7.1% after 24 hours (Feilzer *et al*, 1988).

Cavity properties such as depth can also be related to the extent of microleakage. This is due most likely to the differences in the dentinal tubule diameter and dentinal tubule density at different parts of the dentine, leading to differences in bonding effectiveness of the material to tooth structure and to dentine specifically (Trowbridge, 1987).

Cavity shape is considered to be the factor that relates closely to the stresses involved in a restoration and so to the phenomenon of microleakage. These stresses were shown to be proportional to the contact surface area which bonds to the restoration (Davidson and De Gee, 1984). It was stated that the increase in the ratio of bonded surface to free surface can increase the internal stress within the restoration. The degree of internal stresses, therefore, varies among different class cavities and the highest values can be found in class I and II cavities (Davidson and De Gee, 1984, Dietschi *et al*, 2002).

It is evident from the literature that cavity design varies amongst studies with respect to the dental material being analyzed (Taylor and Lynch, 1993, Hilton, 2002). For example, the authors in one study introduced the beveling of enamel margins to compare the microleakage of composite resins in non-beveled cavities (butt margins) and beveled cavities and found that beveling enamel margins reduced microleakage (Holtan *et al*, 1990).

Another cavity modification was introduced with one and two notches placed at the axial-gingival line angle in class II cavities and found that the notches improved marginal sealing (Coli *et al*, 1993). Moreover, a variety of cavity shapes have also been introduced such as saucer-shaped preparations (Krejci and Lutz, 1990), wedge-shaped class V cavities (Prati and Nucci, 1991), and cylindrical class V cavities (Kamel and Retief, 1990).

Location of the cavities can also be an important factor closely related to the microleakage results obtained. This is because adhesive materials may behave differently against enamel, dentin and cementum, resulting in internal stresses and marginal adaptation differences with resultant microleakage (Frankenberger *et al*, 2005).

2.2.8.4 – Microleakage expression and analysis

The most popular technique for the investigation of restoration sealing is through microleakage studies (Taylor and Lynch, 1992, Gale and Darvell, 1999, Hilton, 2002), in which the uses of dyes for *in vitro* experiments have been dominant. As a result of this work a number of issues concerning methodology reliability and technique sensitivity have arisen. Of particular concern are the issues of microleakage expression and analysis, both of which can affect microleakage results. However, studies that have compared microleakage using a different methodology have found that dye immersion time and different thermocycling techniques did not affect microleakage results (Hilton, 2002).

Thermocycling aims at thermally stressing the junction at the tooth-restoration interface by' subjecting the restored tooth to extreme temperature changes comparable with temperature changes encountered intra-orally (Wahab *et al*, 2003).

2.3 – DENTAL BLEACHING

2.3.1 – Tooth bleaching

To bleach means to remove the color or to whiten the object, so tooth bleaching is a procedure that deals with whitening the teeth by means of bleaching agents. The lightening of the color of a tooth through the application of a chemical agent occurs through the oxidization of the organic pigmentation in the tooth and is referred to as bleaching (Fastanto, 1992).

Bleaching of discolored, pulpless teeth was first described in 1864 (Truman 1864 cited by Dahl and Pallesen, 2003). A variety of medicaments such as chloride, sodium hypochlorite, sodium perborate, and hydrogen peroxide have been used for bleaching either alone or in combination, and with and without heat activation (Howell, 1980).

The observation that carbamide peroxide caused lightening of the teeth was made in the late 1960s by an orthodontist who had prescribed an antiseptic containing 10% carbamide peroxide to be used in a tray for the treatment of gingivitis (Haywood, 1991). The observation was communicated to other colleagues and must be regarded as the beginning of the night guard bleaching era. More than 20 years later, the method describing the use of 10% carbamide peroxide in a mouth guard to be worn overnight for lightening tooth color was published (Haywood and Heymann, 1989).

2.3.2 – Tooth bleaching mechanism

Present-day tooth-bleaching techniques are based upon hydrogen peroxide as the active agent. The hydrogen peroxide is applied directly, or it is produced in a chemical reaction from sodium perborate or carbamide peroxide in solution (Carrillo *et al*, 1998).

Hydrogen peroxide works as a durable oxidizing agent through the formation of free radicals, reactive oxygen molecules and hydrogen peroxide anions. These reactive molecules attack the stains (dark-colored, long chains chromophore molecules) and divide them into smaller and more diffusible molecules. The smaller molecules reflect less light, thus creating a whitening effect. The bleaching agents and their active part (hydrogen peroxide) yield urea which in turn facilitates the bleaching procedure by its high pH. However the effect of the bleaching procedure depends on the concentration of the bleaching agent, the duration and the number of the applications of the agent that come in contact with the stain molecules (Goldstein and Garber, 1995).

2.3.3 – Types of bleaching

2.3.3.1 – Non-vital tooth bleaching

The primary indication for nonvital bleaching is to lighten teeth that have undergone root canal therapy. This discoloration may be as a result of bleeding into the dentin from trauma before the root canal therapy, degradation of pulp

tissue left in the chamber after root canal therapy, or staining from the restorative materials and cements placed in the tooth as part of the root canal treatment (Watts and Addy, 2001).

Non-vital bleaching techniques include an in-office thermocatalytic technique and an out of the office technique referred to as the walking bleach technique (Goldstein, 1997).

2.3.3.1.1 – In-office non-vital bleaching technique

This is a thermocatalytic technique involving the placement of a 35% hydrogen peroxide liquid into the debrided pulp chamber with the acceleration of the oxidation process by the placement of a heating instrument into the pulp chamber in contact with the hydrogen peroxide liquid (Goldstein, 1997). Recently the preference has been to use 30% hydrogen peroxide paste or even gels have been favored as these seem to avoid cervical root resorption which is a common side-effect of this technique (Carrillo *et al*, 1998).

It is essential that a sealing cement such as polycarboxylate or a light-cured glass ionomer cement should be placed over the exposed root canal filling before the application of the bleaching agent to prevent leakage and penetration of the bleaching material in an apical direction with resultant root resorption (Goldstein, 1997).

2.3.3.1.2 – "Walking" bleach technique

The "walking bleach" technique is a non-vital bleaching technique that was introduced in 1961 and involved the placement of a mixture of sodium perborate and water into the pulp chamber that was then sealed off between the patient's visits to the clinician (Spasser 1961 cited by Dahl and Pallesen, 2003). The method was later modified and the water in the mixture was replaced by 30 to 35% hydrogen peroxide, to improve the whitening effect (Nutting and Poe 1963 cited by Dahl and Pallesen, 2003).

An alternative treatment option for a failed, nonvital, "walking bleach" procedure is the technique used in the external vital bleaching procedure (Baratieri *et al*, 1995, Caughman *et al*, 1999).

2.3.3.2 – Vital tooth bleaching

Indications for vital tooth bleaching include intrinsically discolored teeth due to aging, trauma, or drug ingestion (Fastanto, 1992).

Vital bleaching is often indicated for discolored teeth before and after restorative treatment to match shades of the restorative material with that of the natural tooth (Williams *et al*, 1992).

Other indications for external bleaching include a single tooth that has darkened from trauma but is still vital and has a poor endodontic prognosis because of the absence of a radiographically visible canal due to calcific metamorphosis (West, 1997).

Vital tooth bleaching can be performed at home and in the office. Four different approaches for tooth whitening have been recognized and reviewed by Barghi (1998):

(1) Dentist-administered bleaching—the use of a high concentration of hydrogen peroxide (from 35% to 50%) or carbamide peroxide (from 35% to 40%), often supplemented with a heat source (Power bleaching).

(2) Dentist-supervised bleaching—by means of a bleaching tray loaded with a high concentration of carbamide peroxide (from 35% to 40%) that is placed in the patient's mouth for 30 minutes to 2 hours while the patient is in the dental office.

(3) Dentist-provided bleaching— known as "at-home" or "night-guard" bleaching and administered by the patient applying from 5% to 22% solution of carbamide

peroxide in a custom-made tray and normally applied for 15 to 30 minutes twice a day for 10 to 14 days depending on the severity of the stain.

(4) Over-the counter products often based on carbamide peroxide or hydrogen peroxide of various concentrations and placed in a pre-fabricated tray, or by the recently introduced strips, both to be adjusted by the user. Examples of these products include Crest Whitestrips (Proctor & Gamble), containing 6.5% Hydrogen peroxide; and Simply-White Gel (Colgate), containing 5.9% Hydrogen peroxide (Patricia, 2007).

2.3.3.2.1 – In-office vital tooth bleaching (dentist-administered bleaching)

The patient must be cautioned about post-operative sensitivity. This procedure requires rubber dam placement. Vaseline or cocoa butter is placed on the patient's lips and gingival tissues before the application of the rubber dam. The anterior teeth are isolated at times including the first premolars with a heavy rubber dam to provide maximum retraction of the tissues and an optimal seal around the teeth.

Etching of the teeth with 37% phosphoric acid, previously considered a required part of these techniques, is now considered unnecessary (Hall, 1991).

A 35% hydrogen peroxide-soaked gauze or a gel or paste is placed on the teeth. The patient is instructed to report any burning sensation of the lips or gingiva that would indicate a leaking dam and the need to terminate the procedure. The oxidation reaction of the peroxide reaction can be accelerated by applying heat with either a heating instrument or heat can be applied with a metal instrument heated over a flame. The application of heat accelerates the reactivity of the bleaching agent and shortens the treatment time. Effective temperatures that do not produce an undue pulpal reaction are in the range of 125º to 140° F or 52º to 60° C, but it is preferable to use a regulated heat source (Hansen-Bayless and Davis, 1992, Sulieman *et al*, 2004).

There are three heating instruments for utilization in the bleaching procedure currently marketed by Union Broach Company (USA).

1. Union Broach heating paddle, a heating instrument with interchangeable metal tips and good heat regulation.
2. New Image Bleaching Unit, a heat lamp with built-in timer and temperature regulation.

3. The Illuminator, a combination unit with both a heat lamp and a heating paddle.

Activation of the hydrogen peroxide can also be achieved with exposure to an intense light like a plasma arc lamp (Apolite 11, DMDS UK, Canterbury) for 30 minutes for each arch (Sulieman *et al*, 2004).

The use of a CO2 laser is prohibited now because of the risk of soft and hard tissue damage according to the American Dental Association (Wolfgang and Attin, 2007).

Upon completion of the treatment, the teeth are rinsed and the rubber dam is removed. Bleaching treatments are generally provided weekly for two to six treatments, with each treatment lasting 30 to 45 minutes (Leonard and Haywood, 1999).

2.3.3.2.2 – Dentist Prescribed-Home applied bleaching technique

The night guard vital bleaching technique is much less labor intensive and just needs supervision and requires substantially less in-office time. The preparation of the tray is crucial. A conventional impression is made. Incomplete rinsing of the impression can cause a softened surface of the stone, which may result in a nightguard bleaching tray that is slightly small and will irritate the tissues. The cast is trimmed around the periphery to eliminate the vestibule and the base of the cast is trimmed out palatally until a hole is produced. Generally, the cast must be lifted off from the table of the model trimming machine to remove the

vestibule successfully without damaging the teeth. The cast is allowed to dry and any significant undercuts are blocked out using a block-out material such as putty, clay or a light-activated spacer material (Baker *et al*, 2007**,** Roberson *et al*, 2002).

The nightguard is then formed on the prepared cast of the patient's teeth using a heat-vacuum-forming machine (Baker *et al*, 2007**,** Roberson *et al*, 2002). After the machine has warmed up for 10 minutes, a sheet of 0.020 to 0.035 inch (0.75 to 1.5 mm) thick soft vinyl nightguard material is inserted and allowed to soften by heat until it sags approximately 1 inch over the cast. The top portion of the machine is closed slowly and gently and the vacuum is allowed to form the heat-softened material around the cast. After sufficient time has lapsed for adaptation of the material, the machine is turned off and the material is allowed to cool (Baker *et al*, 2007**,** Roberson *et al*, 2002).

A pair of scissors or a number 11 surgical blade in a Bard-Parker handle is used to trim the nightguard in a smooth, straight cut about 3 to 5 mm from the most apical portion of the gingival crest of the teeth (facially and lingually). The excess material is removed using a sharp, curved pair of scissors; the horseshoe-shaped nightguard is removed from the cast (Baker *et al*, 2007**,** Roberson *et al*, 2002).

The facial edges of the nightguard are trimmed in a scalloped design, following the outline of the free gingival crest. Scalloping of the lingual surface is optional, because the bleaching material is applied primarily to the facial aspects of the teeth. Alternately on the lingual aspect, the nightguard may be trimmed apically to within 2 mm of the free gingival crest in a smooth, horseshoe-shaped configuration.

A scalloped design of the night guard is preferred because it allows the tray to cover only the teeth and prevents entrapment of the bleaching material between the gingival tissue and the nightguard (Sophia and Aaron, 2009). The nightguard is completed and delivered to the patient.

The nightguard is inserted into the patient`s mouth and evaluated for adaptation, rough edges, or blanching of the tissues. The occlusion on the nightguard is evaluated with the patient closing his jaw in maximum intercuspation. If the patient is unable to obtain a comfortable occlusion because of premature posterior tooth contacts, the nightguard is trimmed to exclude coverage of the terminal posterior teeth as needed to allow optimal tooth contact in maximum intercuspation. In addition, if no lingual scalloping is done, the edges of the nightguard on the palate should terminate in the grooves or valleys where possible, rather than on the heights of soft-tissue contours such as in the area of the incisive papilla (Baker *et al*, 2007**,** Roberson *et al*, 2002).

A 10% to 15% carbamide peroxide- bleaching material generally is recommended for this bleaching technique (Dahl and Pallesen, 2003). Commercial bleaching products are available as both clear gels and white pastes. Carbamide peroxide degrades into 3% hydrogen peroxide which is the active ingredient and 7% urea. Bleaching materials containing carbopol are recommended because it thickens the bleaching solution and extends the oxidation process (Williams *et al*, 1992).

Based on numerous research studies, carbamide peroxide bleaching material appears to be safe and effective for home bleaching when administered by or under the supervision of a dentist (Tredwin *et al*, 2006).

The patient is instructed as regards the application of the bleaching gel or paste into the nightguard. A thin bead of material is extruded into the nightguard along the facial aspects corresponding to the area of each tooth to be bleached. Usually only the anterior 6 to 8 teeth are bleached. The clinician should review proper insertion of the nightguard with the patient. After inserting the nightguard, excess material is wiped from the soft tissue along the edge with a soft-bristled toothbrush. No excess material should be allowed to remain on the soft tissues because of the potential for gingival irritation. The patient should be informed not to drink liquids or rinse during the treatment, and to remove the

nightguard for meals and oral hygiene procedures (Hao *et al*, 2008, Baker *et al*, 2007**,** Roberson *et al*, 2002).

If the nightguard is worn at night, a single application of bleaching material at bedtime is indicated. The nightguard is removed in the morning cleaned under running water with a soft tooth-brush, and stored. Total treatment time using an overnight approach is usually 1 to 2 weeks.

It is recommended that only one arch be bleached at a time, beginning with the maxillary arch. Bleaching the maxillary arch first allows the untreated mandibular arch to serve as a standard for comparison (Alonso and Balboa, 2006).

2.3.3.2.3 – Over-the counter products

These products typically contain low levels of a whitening agent such as 3% to 6% hydrogen peroxide, which is self-applied to the teeth via gum shields, strips, or paint-on product formats. These products typically require twice per day application for up to 2 weeks depending on the intensity of the stain (Andrew, 2007, Mielczarek *et al*, 2008).

These products were created to avoid the use of trays for the application of the bleaching agent. Adhesive strips containing bleaching agents are bonded to the anterior teeth, and they release the active ingredient during relatively short time periods of 5 to 60 minutes, once or twice a day. The active ingredient is hydrogen peroxide (HP) in low concentrations of 5% to 14% (Donly *et al*, 2007).

Studies have demonstrated that there is an increase in the whitening effect when the strips are used for 28 days compared to when they are only used for 14 days, and more importantly the whitening effect could be maintained for 2 years (Gerlach and Barker, 2004).

CHAPTER THREE

AIM AND OBJECTIVES

3.1 – Aim

To assess the effect of bleaching on the marginal integrity of Class V composite resin restorations.

3.2 – Objectives

To determine the effect of a 6% hydrogen peroxide over the counter and a 38% hydrogen peroxide in-office vital bleaching treatment products on the microleakage of Class V composite restorations.

3.3 – Null Hypothesis

There is no significant difference in the microleakage of bleached and conventional class V composite resin restorations.

CHAPTER FOUR

MATERIALS & METHODS

4.1 – Study Design

This is an *in vitro* experimental study.

4.2 – Sample size

60 extracted human molar teeth were used in this study.

4.3 – Inclusion criteria

Only non-carious and non-restored human molar teeth extracted for orthodontic or prophylactic purposes were used in this study.

4.4 – Exclusion criteria

- Teeth which were extracted due to dental caries.
- Teeth with restorations or cracks.

4.5 – Materials

- Z 100 composite resin (figure 4.1): A microhybrid composite by 3M ESPE, USA. Filler loading: 72% by weight and 66% by volume. Polymerization Shrinkage: 2.8%. Particle size: 3.5-0.01 μm. Resin: bis-GMA and TEGDMA.

Figure 4.1: Z I00 Composite resin restorative material

- Hydrogen peroxide 6 % in an aqueous solution.

- 38% hydrogen peroxide Opalescence Boost tooth whitening system (ULTRADENT, USA)

4.6 – Procedure

All the extracted teeth meeting the inclusion criteria were cleaned using a rubber cup and pumice to remove the surface debri and stains. The teeth were disinfected in a 0.5% chloramine T solution and subsequently stored in a 0.9% saline solution (Loguercio *et al*, 2004).

The roots of all the teeth were removed with a model trimmer using water as a coolant. A cavity was prepared in the root apices that were sectioned, with a round carbide bur (C1204008, Horico Germany) in a slow speed hand-piece. Each cavity was rinsed with water, dried with air and conditioned with GC dentine conditioner. The cavities were then filled with resin modified glass ionomer cement and sealed with Vitremer (3M ESPE, USA).

Class V cavities were prepared on the facial surfaces of the teeth using an F 0111 fissure bur (Dentsply, Germany) in a high speed hand-piece under

copious water irrigation. The dimensions of the preparation were 3 mm in width and 2 mm in depth. The preparation was 1.5 mm above the cemento-enamel junction (CEJ) and 1.5 mm below the cemento-enamel junction (CEJ) to include enamel and dentin margins in the preparation (Figure 4.2). Burs were replaced after every eight preparations (Hilton *et al*, 1997).

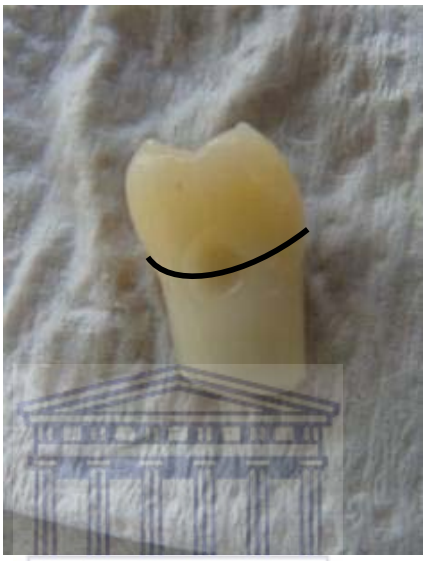

Figure 4.2: Facial surface of the tooth illustrating cavity preparation and cemento-enamel junction

The teeth were restored with Scotchbond Multi-Purpose Plus (3M ESPE, USA) using a three-step dentin bonding system with the first application being that of phosphoric acid 35% (ULTRADENT, USA) as etchant to the cavity for 40 seconds then washed away with a water-air syringe for 5 seconds and dried with air for 5 seconds. Secondly an application of one layer of primer then air dried for 5 seconds and light cured with a halogen light curing unit (Demetron LC, sdsKerr, USA) for 5 seconds. Thirdly an application of one layer of adhesive then air dried for 5 seconds and light cured with a halogen light curing unit (Demetron LC, sdsKerr, USA) for 10 seconds.

The composite restorative material Z100/Adper Scotchbond (3M ESPE, USA) was placed in 1 mm increments and a conventional curing light (Demetron LC, sdsKerr, USA) was used to cure each increment for 40 seconds prior to the

placement of the next increment with the tip of the curing light being held 5 mm away from the restoration at all times during the curing process.

All restorations were finished and polished with aluminum oxide-coated flexible Sof-Lex discs, (3M ESPE, USA) (Loguercio *et al*, 2004). All the teeth were stored in distilled water at 37 °C for 7 days.

The teeth were randomly divided into three groups (n=20), and each group was marked with a different colored nail varnish. The first group was the control group, the second and third groups were the experimental groups.

The control group was stored in distilled water at 37º C until the end of the study.

The experimental group one was bleached with 6% hydrogen peroxide for 30 minutes twice daily for 14 days simulating the effect of Crest Whitestrips (Procter & Gamble CO., Cincinnati, OH, USA).

The experimental group two was bleached with 38% hydrogen peroxide Opalescence Boost tooth whitening system (ULTRADENT, USA). After mixing the bleaching agent according to the manufacturer`s instructions, the gel was applied to the whole filling including the margins for 20 minutes in three sessions. The bleaching was carried out twice and the interval between the two applications was five days to simulate the clinical situation.

The experimental groups were stored in distilled water at 37º C. except during the bleaching treatment.

In order to prevent dye penetration into the dentinal tubules or the lateral canals adjacent to the restorations, the teeth were coated with two layers of nail varnish except for an area approximately 2 mm around the margins of the restorations (Loguercio *et al*, 2004). The nail varnish was allowed to dry for 12 hours before thermocycling the teeth.

In an attempt to simulate the temperature changes that take place in the oral cavity, the specimens were subjected to thermal cycling (Figure 4.3). All specimens were subjected to thermocycling according to the International Organization for Standardization (ISO) TR11405 standard of 500 cycles, at 5° to 55 °C, with a 15 second dwell time (Bitter *et al*, 2008, Loguercio *et al*, 2004,) in a buffered (pH 7) 0.5% methylene blue solution dye (Figure 4.4).

Figure 4.3: Thermal cycling

Figure 4.4: Specimens after Thermal cycling

After removal from the dye, the specimens were thoroughly washed under tap water for 10 minutes. The specimens were transferred to specimen bottles containing distilled water until the time of sectioning. The nail varnish was removed with an acetone solution and all the specimens were again cleaned with water (Figure 4.5). The specimens were embedded in a slow setting epoxy resin and allowed to set overnight.

Figure 4.5: Specimen after cleansing

Each restoration was sectioned with a 0.35 mm thick blade in a diamond disk cutter water-cooled microtome (Struers Minitom, Germany) (Figure 4.6) through the center of the restoration mesiodistally parallel to the long axis of the tooth (Klukowska *et al*, 2008) (Figure 4.7). Three sections per restoration of approximately 0.5 mm thickness provided six surfaces for evaluation of microleakage at the tooth-restoration interface.

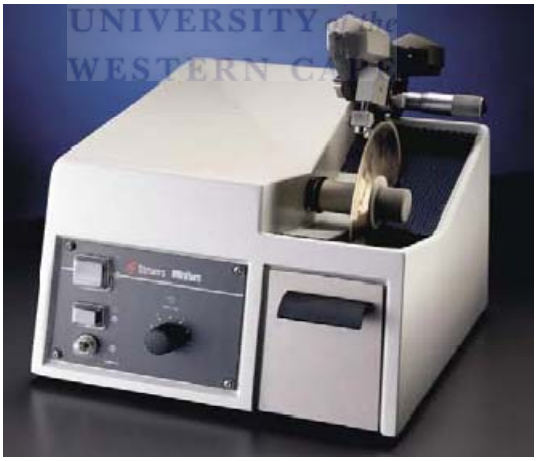

Figure 4.6: Struers Minitom

Microleakage was evaluated at the margins of the class V composite resin restorations under a stereomicroscope (Wild, Heerbrugg Switzerland) (Figure 4.8) using ten times magnification by two previously calibrated examiners. Each examiner measured the microleakage of the three sections (six surfaces) of the

tooth-restoration interfaces; thus, each section was scored four times and each restoration was scored 12 times by the two examiners.

Figure 4.7: Specimen for microleakage evaluation

Any discrepancy between the two examiners was re-evaluated by both until a consensus score was reached.

Figure 4.8: Stereomicroscope

The severity of dye penetration was analyzed using a zero to three-scale scoring system (Loguercio *et al*, 2004). Figure 4.9 is a diagrammatic sketch of the tooth-restoration complex illustrating the basis of the scoring system applied.

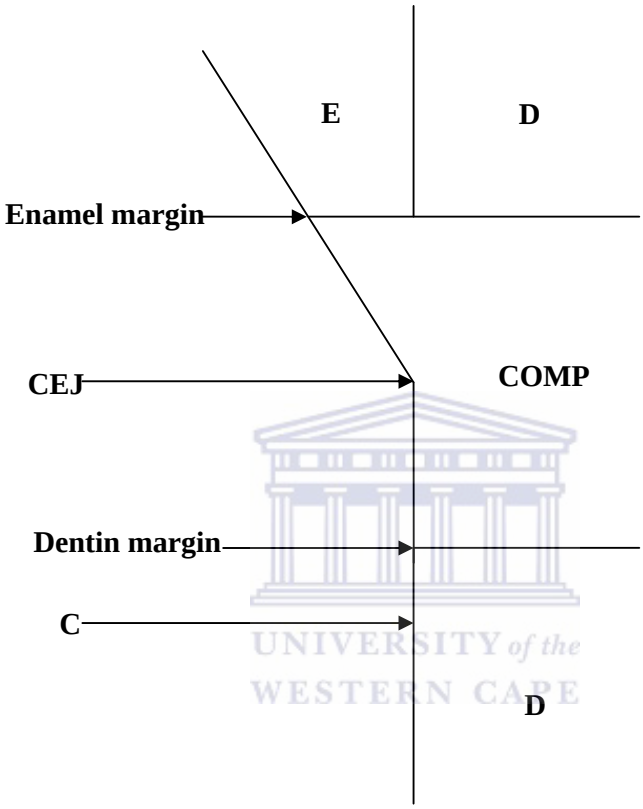

Figure 4.9: Diagrammatic sketch showing a section through the class V composite restoration.

CEJ: cementoenamel junction**, E:** enamel**, D:** dentin, **COMP:** composite **and C:** cementum**.**

For the enamel margin as depicted in the diagrammatic sketch in figure 4.10, the scoring for leakage was as follows:

0 = no penetration.
1 = leakage up to half the enamel thickness.
2 = leakage to the full enamel thickness.
3= leakage beyond the dentinoenamel junction.

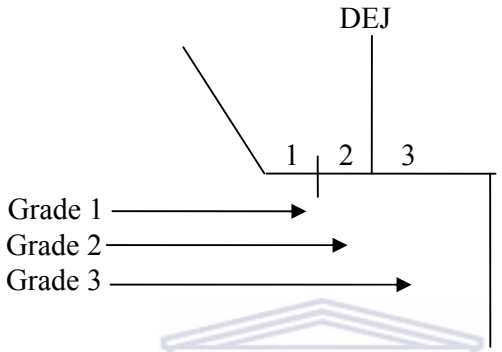

Figure 4.10: Diagrammatic sketch of cross section through the class V cavity showing how the leakage was scored at the enamel margin.

For the dentin margin as depicted in the diagrammatic sketch in figure 4.11, the scoring for leakage was as follows:

0 = no dye penetration.
1 = dye penetration up to one half of the depth of the cavity.
2 = dye penetration more than one half of the depth of the cavity.
3 = dye penetration up to the axial wall of the cavity.

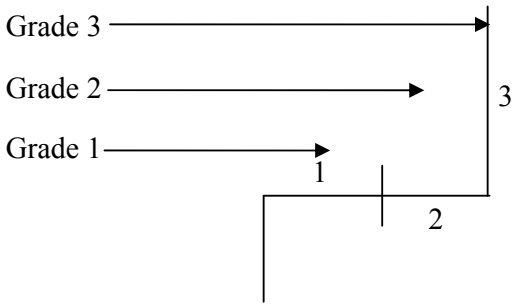

Figure 4.11: Diagrammatic sketch of cross section through the class V cavity showing how the leakage was scored at the dentin margin.

The scoring from the independent observers and the final consensus scores were tabulated in an excel spreadsheet (Appendix 1).

The data was analyzed using a commercially available statistical software package (SPSS 18.0, SPSS Inc.).

The original scores were supplied to the statistician. For a comparison of the microleakage, the median of the 12 dye penetration data measured for enamel and dentin separately on each restoration was recorded. The data was analyzed using a Kruskal-Wallis one way ANOVA on ranks (significance at $p \leq 0.05$) to find if there were any differences that were statistically significant between the groups. The Mann- Whitney *U* test was then used for pair-wise comparison between the groups and Wilcoxon signed ranks test for comparison between enamel and dentin margins.

CHAPTER FIVE

RESULTS

5.1 – Microleakage

5.1.1 – Microscopic Findings

There was a varying degree of dye penetration along the enamel and the dentin margins of the three groups. In some sections the dye penetrated not only along the restorations, but also penetrated into the adjacent dentinal tubules. Dye penetration was more severe in the restorations with dentine or cementum margins as compared to the restorations with enamel margins. No voids were observed between the different increments of the restorative materials and between the bonding agent and the restorative material.

5.1.2 – Microleakage scoring

The scoring criterion of 0, 1, 2 and 3 was used to score the microleakage at the enamel and dentin cementum margins. The microleakage score for each specimen was recorded in an excel spreadsheet and appears in appendix 1.

For the enamel margin scoring for leakage was as follows:

0 = no penetration.
1 = leakage up to half the enamel thickness.
2 = leakage to the full enamel thickness.
3= leakage beyond the dentinoenamel junction.

For the dentin margin scoring for leakage was as follows:

0 = no dye penetration.
1 = dye penetration up to one half of the depth of the cavity.
2 = dye penetration more than one half of the depth of the cavity.
3 = dye penetration up to the axial wall of the cavity.

The total number of each score in each group was calculated and is summarized in Table 5.1 for the enamel margins and in Table 5.2 for the dentine margins. These results are graphically illustrated in figure 5.1 and 5.2 respectively.

GRADES	CONTROL GROUP	FRIST EXPERIMENTAL GROUP	SECOND EXPERIMENTAL GROUP
GRADE 0	26	22	0
GRADE 1	72	60	57
GRADE 2	16	36	57
GRADE 3	6	2	6
TOTAL	120	120	120

Table 5.1 Total number of microleakage scores for each group: ENAMEL

GRADES	CONTROL GROUP	FRIST EXPERIMENTAL GROUP	SECOND EXPERIMENTAL GROUP
GRADE 0	0	0	0
GRADE 1	13	0	13
GRADE 2	29	43	50
GRADE 3	78	77	57
TOTAL	120	120	120

Table 5.2 Total number of microleakage scores for each group: DENTIN

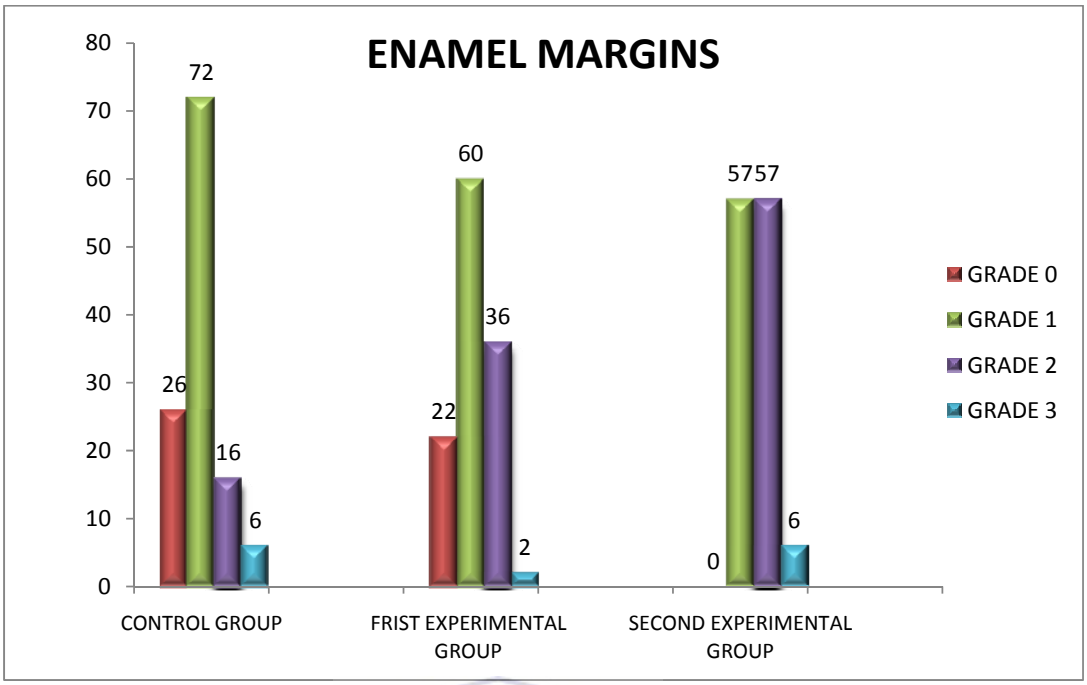

Figure 5.1 Enamel Microleakage Scores for Each Group

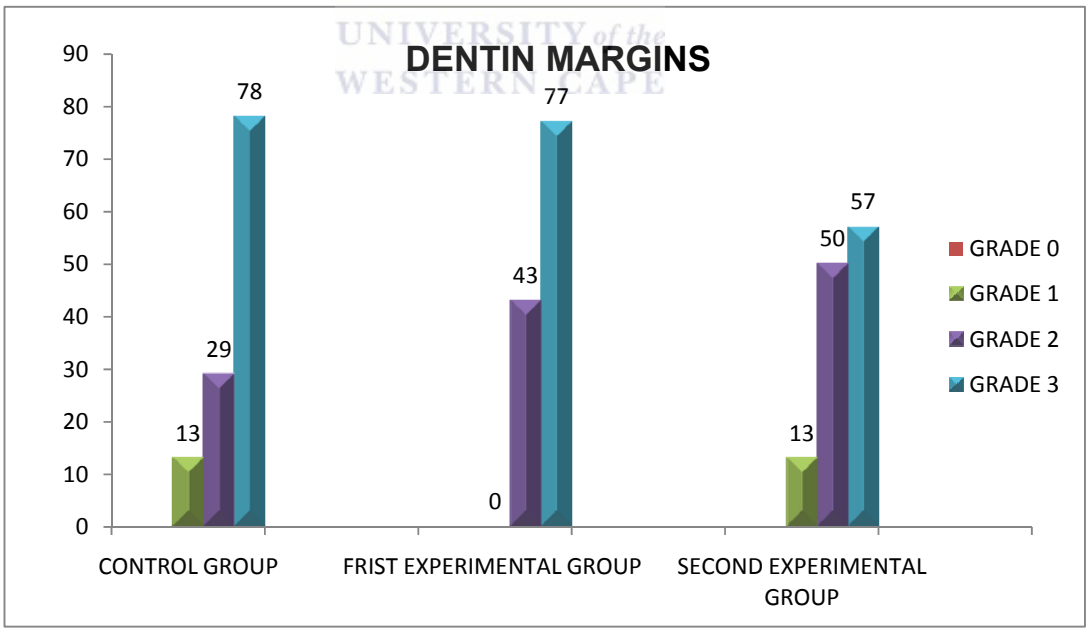

Figure 5.2 Dentin Microleakage Scores for Each Group

A Kruskal-Wallis analysis of variance (ANOVA) test was carried out to investigate if statistically significant differences existed between the three groups at a significance level of $p \leq 0.05$ for both enamel and dentin margins. A summary of the means, number of sections scored the standard deviation, the median as well as the minimum and maximum values for microleakage at the enamel and dentin margins are represented in Table 5.3 and 5.4 respectively.

ENAMEL						
Smallest N for any variable: 360						
	Means	N	Std. Dev.	Minimum	Median	Maximum
C. group	0.93	120	0.613	0	1	2.5
F.E group	1.13	120	0.559	0	1	2
S.E group	1.60	120	0.503	1	1.8	2.5

Table 5.3 Summary of means, number of sections scored, standard deviation, median, minimum and maximum values for microleakage at the enamel margins.

DENTIN						
Smallest N for any variable: 360						
	Means	N	Std. Dev.	Minimum	Median	Maximum
C. group	2.53	120	0.638	1	3	3
F.E group	2.70	120	0.470	2	3	3
S.E group	2.35	120	0.651	1	2.3	3

Table 5.4 Summary of means, number of sections scored, standard deviation, median, minimum and maximum values for microleakage at the dentin margins.

The results of the Kruskal-Wallis test showed that there was a statistically significant difference between the three groups for the enamel margins but there was no statistically significant difference between the three groups as regards the dentin margins and this is illustrated in table 5.5.

Ranks

	Group	N	Mean Rank
Enamel margins groups	1	20	22.88
	2	20	27.60
	3	20	40.03
	Total	60	
Dentin margins groups	1	20	30.00
	2	20	33.95
	3	20	25.84
	Total	60	

	Enamel margins groups	Dentin margins groups
Chi-Square	12.225	2.801
Df	2	2
Asymp. Sig.	**.002**	**.247**
Test Statistics	**Statistically significant**	**Statistically not significant**

Table 5.5 Kruskal-Wallis test illustrating a statistically significant difference between the three groups for the enamel margins ($p \leq 0.05$) but no statistically significant difference between the 3 groups for the dentin margins ($p \leq 0.05$).

Once it was established that there was a statistically significant difference between the groups as regards the enamel margins, a Mann-Whitney *U* test was carried out for a pair-wise comparison to determine which group differed from the others at a significance level of **p≤ 0.05**.

Ranks

	Group	N	Mean Rank	Sum of Ranks
Enamel margins groups	1	20	18.80	376.00
	2	20	22.20	444.00
	Total	40		

	Enamel margins groups
Mann-Whitney U	166.000
Wilcoxon W	376.000
Z	-1.041
Asymp. Sig. (2-tailed)	**.298**
Test Statistics	**Differences statistically not significant**

Table 5.6 Mann-Whitney *U* test for differences between the control and the first experimental group.

There was no statistically significant difference between the control group and the first experimental group for the enamel margins. **(p≤0.05)**

Ranks

	Group	N	Mean Rank	Sum of Ranks
Enamel margins groups	1	20	14.58	291.50
	3	20	25.71	488.50
	Total	40		

	Enamel margins groups
Mann-Whitney U	81.500
Wilcoxon W	291.500
Z	-3.345
Asymp. Sig. (2-tailed)	**.001**
Test Statistics	**Differences statistically significant**

Table 5.7 Mann-Whitney *U* test for differences between the control group and the second experimental group.

However there was a statistically significant difference between the control group and the second experimental group **(p≤ 0.05)** implying that the leakage was worse in the second experimental group.

Ranks

	Group	N	Mean Rank	Sum of Ranks
Enamel margins groups	2	20	15.90	318.00
	3	20	24.32	462.00
	Total	40		

	Enamel margins groups
Mann-Whitney U	108.000
Wilcoxon W	318.000
Z	-2.448
Asymp. Sig. (2-tailed)	**.014**
Test Statistics	**Differences statistically significant**

Table 5.8 Mann-Whitney *U* test for differences between the first and second experimental groups.

There was a statistically significant difference between the first and second experimental groups **(p≤ 0.05)** implying that the second experimental group leaked more than the first experimental group.

Wilcoxon Signed Ranks Test was carried out for a pair-wise comparison between the median of the enamel and dentin margins for every group.

	MDMC – MEMC	MDME1 - MEME1	MDME2 - MEME2
Z	-3.819[a]	-3.866[a]	-2.658[a]
Asymp. Sig. (2-tailed)	**.000**	**.000**	**.008**

Table 5.9 Wilcoxon signed ranks test for differences between the enamel and dentin margins for every group.

MDMC/ MEMC: Median Dentin/ Enamel Margins Control group**,**

MDME1-2/ MEME1-2: Median Dentin/Enamel Margins Experimental group 1/ 2.

Wilcoxon signed ranks test showed that there was a statistically significant difference between the dentin and enamel margins **(p≤ 0.05)** in the control and both experimental groups implying that the dentin margins leaked more than the enamel margins in all 3 groups.

CHAPTER 6

DISCUSSION

6.1– Microscopic findings

As mentioned in chapter five, there were no voids observed between the different increments of the restorative material and between the bonding agent and the restorative material. The key to avoid the presence of voids between the bonding agent and the composite resin restorative material is when applying the bonding agent. To remove excess bonding agent from the cavity by gently blowing air for five seconds to avoid making a thick layer or pooling that in future may lead to gap formation. The key to avoid the presence of voids between the different increments of the composite resin restorative material is the layering technique (Figueiredo *et al*, 2003) with separate curing and with close adaptation of the layers against the walls of the cavity and to each other after curing (Giachetti *et al*, 2006).

6.2– Microleakage

6.2.1– Enamel margins

In the control group and the first experimental group (Hydrogen peroxide 6 % in an aqueous solution), there were 26 specimens in the control group (21.67%) and 22 specimens in the first experimental group (18.33%) that had a score of 0.

This is a score indicating no evidence of microleakage while the second experimental group (38% hydrogen peroxide Opalescence Boost tooth whitening system (ULTRADENT, USA) had no specimens with a score of zero implying that whatever was used in experimental group two must have affected

the tooth-restoration interface to some extent that resulted in all the specimens showing some sign of leakage.

This is supported by the Mann-Whitney *U* test in table 5.7 and table 5.8 that show a statistically significant difference between the control group and the first experimental group from the second experimental group at a p value of less than 0.05.

- p value between group 1 and 3 = 0.001
- p value between group 2 and 3 = 0.014

Grades of 1, 2 and 3 reflect varying degrees of leakage. Table 6.1 reaffirms that most tooth-composite restoration interfaces leak to some extent with 78% in the control group, 82% in the first experimental group and 100% in the second experimental group showing signs of microleakage at the enamel margin at the tooth-restoration interfaces.

GRADES	CONTROL GROUP	FRIST EXPERIMENTAL GROUP	SECOND EXPERIMENTAL GROUP
GRADE 0	26 (21.67%)	22 (18.33%)	0 (0%)
GRADE 1	72 (60%)	60 (50%)	57 (47.50%)
GRADE 2	16 (13.33%)	36 (30%)	57 (47.50%)
GRADE 3	6 (5%)	2 (1.67%)	6 (5%)
TOTAL	120 (100%)	120 (100%)	120 (100%)

Table 6.1 Total number of microleakage scores for each group: ENAMEL

6.6.2– Dentin margins

From table 5.2 as there were no specimens with a score of zero, it implies that all composite restoration-tooth interfaces in this study leaked surprisingly with the worst leakage (score of 3) registered in the control group with 78 specimens (65% of specimens) and the first experimental group with 77 specimens (64.17%) of all specimens in the group.

Statistically there was no significant difference in leakage between the three groups as regards leakage at the interface of the tooth-restoration at the dentine margin. This is substantiated by the Kruskal-Wallis test which revealed no statistically differences between the groups (2 experimental and the control group) within p value = 0.247 which is **> 0.05** (Table 5.5)

The finding in table 6.2 indicates that all three groups scored the largest number ranging from 48% to 65% of grade three scores implying that the dye penetrated up-to the axial wall of the cavity and the marginal seal at the dentin cementum margin is non existant.

GRADES	CONTROL GROUP	FRIST EXPERIMENTAL GROUP	SECOND EXPERIMENTAL GROUP
GRADE 0	0 (0%)	0 (0%)	0 (0%)
GRADE 1	13 (10.83%)	0 (0%)	13 (10.83%)
GRADE 2	29 (24.17%)	43 (35.83%)	50 (41.67%)
GRADE 3	78 (65%)	77 (64.17%)	57 (47.50%)
TOTAL	120 (100%)	120 (100%)	120 (100%)

Table 6.2 Total number of microleakage scores for each group: DENTIN

The analysis of the present study indicates microleakage from the worst leakage to the least leakage in the interfaces of restorations with enamel margins to be described in the sequence depicted in table 6.3:

Enamel Margins	Microleakage
Second Experimental Group(38% hydrogen peroxide Opalescence Boost tooth whitening system (ULTRADENT, USA)	Worst leakage
First Experimental Group (Hydrogen peroxide 6 % in an aqueous solution)	↓
Control Group	Least leakage

Table 6.3 Ranking of the groups according to the severity of microleakage

This ranking was substantiated by the statistical analysis where in the case of the enamel margins, the second experimental group differed significantly

statistically from the other groups however; there was no statistically significant difference between the control and the first experimental group.

In this study, the results from the statistical analysis showed that bleaching treatment with 38% hydrogen peroxide in the second experimental group adversely affected the marginal seal at the tooth-restoration interface for the enamel margins as evidenced by increased microleakage and this is in accordance with previous studies conducted by (Crim, 1992 (a), Barkhordar *et al*, 1997, Waite *et al*, 1998, Shinohara *et al*, 2001, White *et al*, 2003, Ulukapi *et al*, 2003, Turkun and Turkun, 2004) who also concluded that bleaching treatment may adversely affect the marginal integrity and increase the microleakage of the composite resin restorations.

However other studies done by (Crim, 1992 (b), Pioch *et al*, 2002, Klukowska *et al*, 2008, White *et al*, 2008) indicated that bleaching treatment did not affect the marginal integrity. This study found that as regards the dentin margin bleaching treatment did not affect the microleakage at the interface of the restoration and the tooth as they all leaked with no statistically significant differences between the 3 groups. This may explain the finding that the same authors found conflicting results in different studies as regards the effect of bleaching agents on the marginal integrity of composite restorations and the resultant microleakage.

According to the manufacturers' Z100 has volumetric polymerization shrinkage values of 2.8%, the poor sealing ability of Z100 may also have contributed to the leakage observed as a result of the higher polymerization shrinkage values of the restorative material.

Enamel has been regarded as a reliable substrate for bonding (Yazici *et al*, 2004), this fact is supported by the finding that microleakage was worse in the dentin margins compared to the enamel margins (table 5.9) in addition the bleaching treatment with 38% hydrogen peroxide increased the microleakage in the enamel margin in this present study.

The results of the statistical analysis showed there was a statistically significant difference between the microleakage in the enamel and the dentin/cementum margins, (p value ranged from 0.000 to 0.008) supporting the assumption that the marginal seal and bonding ability is much better in the enamel than in the dentin. (Table 5.9)

The in-office power bleaching with 38% hydrogen peroxide Opalescence Boost tooth whitening system (ULTRADENT, USA) increased the leakage at the tooth-restoration interface at the enamel margin more than the bleaching with 6% hydrogen peroxide simulating the effect of Crest Whitestrips (Procter & Gamble CO., Cincinnati, OH, USA) as indicated by the results of the statistical analysis in table 5.8.

For the dentin margin, the dentin is not a good substrate for adhesion and bonding because of its low inorganic content 75% compared to enamel 95% (Yazici *et al*, 2004). Difficulty in obtaining good adhesion to dentine or cementum was observed in this study and the leakage was similar in the three groups indicating that the bleaching treatment played a minor or no role in increasing the leakage at the dentin margin indicated by the results of the statistical analysis in table 5.6.

A study done by Crim, 1992 (b), showed that the pre-restorative bleaching did not affect the marginal seal of subsequently placed restorations, but in this study the bleaching treatment was done after the placement of composite resin restorations as it was more clinically relevant to study the effect of bleaching treatment on already placed restorations. Crim, 1992 (a) when doing the same procedure found the carbamide peroxide adversely affect the marginal seal of the composite resin restorations.

CHAPTER 7

LIMITATIONS OF THE STUDY

Laboratory studies attempt to reproduce clinical situations but do not entirely reflect variables encountered with the *in vivo* performance of the materials. The main limitation of this study relates to the relevance of *in vitro* studies in predicting the clinical performance of the materials being tested. Extrapolating the data of *in vitro* observations to the clinical situation is often unreliable and should be done with caution for the following reasons according to Swift *et al*, 1995:

• Tests of this type do not take into account the three-dimensional nature of tooth preparations, and thus underestimate the effects of polymerization shrinkage.

• Other factors that can affect the results may include age and storage conditions of specimens, location and depth of the dentine, thermocycling procedures and the type and duration of the loading forces.

Pashley, (1990) reported that the results of an *in vitro* microleakage study should be viewed as a theoretical maximum level of leakage that may be expected *in vivo*.

CHAPTER 8

CONCLUSIONS AND RECOMMENDATIONS

8.1 – CONCLUSIONS

This *in vitro* study evaluated the effect of bleaching on the marginal integrity of Class V composite resin restorations. The null hypothesis was rejected for part of the study because the results showed a statistically significant difference in the microleakage of the 3 groups at the enamel margin. However at the dentin margin, the null hypothesis was accepted as there was no statistically significant difference in the leakage between the 3 groups (bleached and conventional).

From the conditions set up for this study, several conclusions can be drawn:

- Z 100 composite resin restorations showed significant microleakage when the margins were in enamel in the control group.

- The leakage was worse at the dentin margins in all three groups.

- The in-office power bleaching treatment 38% hydrogen peroxide Opalescence Boost tooth whitening system (ULTRADENT, USA) used in this study increased the microleakage at the tooth-restoration interface in enamel margins.

- To simulate the effect of over the counter products 6% hydrogen peroxide Crest Whitestrips (Procter & Gamble CO., Cincinnati, OH, USA) a 6% hydrogen peroxide in aqueous solution was used in this study for bleaching one of the groups, the statistical analysis showed that there was no statistically significant difference between the control group

(unbleached) and the group bleached with 6% hydrogen peroxide (first experimental group).

- The in-office power bleaching with 38% hydrogen peroxide increased the leakage compared to the 6% hydrogen peroxide bleaching which did not significantly increase the microleakage of the composite restorations after bleaching at the enamel margins.

8.2 – RECOMMENDATIONS

The results of the study are *in vitro* data and definite conclusions should not be drawn until long term *in vivo* studies are completed. More research is needed in the future, especially concerning the Z 100 composite resin material, dentin surface moisture and the adhesive systems used.

Concerning microleakage tests, it is also needed to determine their real importance and ability to predict the clinical performance of the restorative materials. If this importance is confirmed, it is necessary to clarify the mechanism of dye penetration in the adhesive interface, and to improve the methodology to avoid the great variability of results.

This study concluded that bleaching treatment with 38% hydrogen peroxide adversely affected the marginal integrity at the tooth-restoration interface in enamel margins so the study recommends delaying of composite resin restorations if 38% hydrogen peroxide is going to be used for the bleaching treatment or changing the already placed restorations after the bleaching treatment with 38% hydrogen peroxide.

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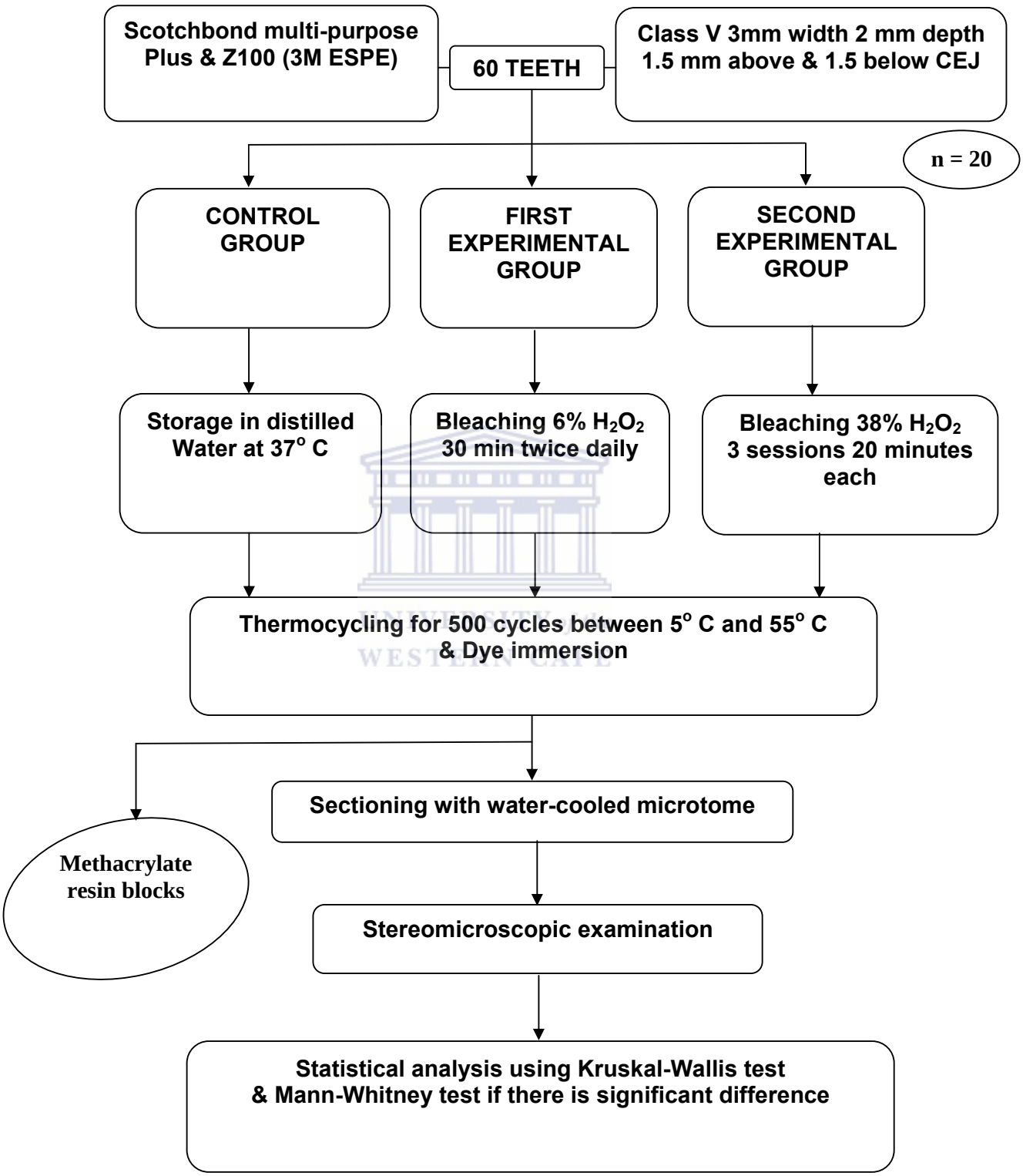
APPENDIX 1
STUDY FLOWCHART



APPENDIX 2
MICROLEAKAGE SCORES

CONTROL GROUP

	ENAMEL MARGIN						DENTIN MARGIN					
	Surface a			Surface b			Surface a			Surface b		
Tooth No	Sec 1	Sec 2	Sec 3	Sec 1	Sec 2	Sec 3	Sec 1	Sec 2	Sec 3	Sec 1	Sec 2	Sec 3
1	1	1	1	1	1	1	3	3	3	3	3	3
2	1	1	1	2	1	1	3	2	2	3	2	2
3	1	2	2	1	1	1	3	3	3	3	3	3
4	1	1	1	1	1	1	3	3	2	3	3	2
5	1	1	1	1	1	1	3	3	3	3	3	3
6	1	1	2	1	1	2	1	2	3	1	1	3
7	0	0	1	0	0	1	3	3	3	3	3	3
8	0	0	0	0	0	0	1	1	1	1	1	1
9	1	0	0	1	0	0	3	3	3	3	3	3
10	0	0	1	1	1	1	2	3	3	3	3	3
11	0	2	1	0	2	1	3	2	2	3	2	2
12	3	3	2	3	2	2	1	2	2	1	2	2
13	1	1	1	3	1	2	3	3	3	3	3	3
14	3	2	1	1	1	1	1	1	2	2	2	3
15	3	2	2	1	1	2	2	3	3	2	2	2
16	1	1	2	1	1	2	2	2	2	2	3	3
17	1	1	1	1	1	1	3	3	3	3	3	3
18	1	0	0	1	1	1	3	3	3	3	3	3
19	1	0	0	1	0	0	3	3	3	3	3	3
20	1	0	1	1	0	1	3	3	3	3	3	3

FIRST EXPERIMENTAL GROUP

Hydrogen peroxide 6 % in an aqueous solution

	ENAMEL MARGIN						DENTIN MARGIN					
	Surface a			Surface b			Surface a			Surface b		
Tooth No	Sec 1	Sec 2	Sec 3	Sec 1	Sec 2	Sec 3	Sec 1	Sec 2	Sec 3	Sec 1	Sec 2	Sec 3
1	2	2	2	2	2	2	3	3	3	3	3	3
2	1	1	1	1	1	1	2	2	2	2	2	2
3	1	1	2	1	1	2	2	2	2	2	2	2
4	2	0	0	1	1	0	3	3	3	3	3	3
5	0	0	1	0	1	1	3	3	2	3	3	2
6	0	0	0	0	0	1	2	3	3	3	3	2
7	2	2	2	2	2	2	2	3	3	3	3	2
8	2	0	1	2	1	0	2	2	3	2	2	3
9	0	0	0	3	1	1	3	3	3	3	3	3
10	3	2	1	0	1	2	3	2	3	2	3	3
11	1	1	2	2	1	2	3	3	3	2	3	3
12	1	1	2	1	1	1	2	2	2	2	2	2
13	2	1	2	2	2	2	2	2	3	2	2	2
14	1	2	1	1	2	1	2	3	3	3	3	3
15	2	2	0	2	2	0	3	3	3	3	3	3
16	1	1	1	1	1	1	3	3	3	3	3	3
17	1	1	2	1	2	1	3	3	3	3	3	3
18	0	1	1	1	1	1	3	3	3	3	3	3
19	1	0	1	1	0	1	2	2	2	2	2	2
20	1	1	1	1	1	1	3	3	3	3	3	3

SECOND EXPERIMENTAL GROUP

38% hydrogen peroxide Opalescence Boost tooth whitening system

(ULTRADENT, USA)

	ENAMEL MARGIN						DENTIN MARGIN					
	Surface a			Surface b			Surface a			Surface b		
Tooth No	Sec 1	Sec 2	Sec 3	Sec 1	Sec 2	Sec 3	Sec 1	Sec 2	Sec 3	Sec 1	Sec 2	Sec 3
1	1	1	1	1	1	1	2	2	2	2	2	2
2	1	2	2	1	1	2	3	3	3	2	3	2
3	3	3	3	2	1	2	2	1	1	1	1	1
4	1	1	1	1	1	1	3	3	2	2	2	2
5	1	1	1	1	1	1	3	2	2	2	2	2
6	2	2	1	2	2	1	2	2	3	2	2	3
7	2	2	2	1	1	2	3	3	3	3	3	3
8	2	1	1	1	2	2	3	3	3	1	3	2
9	3	2	2	2	2	2	1	1	1	1	1	1
10	2	2	2	2	2	2	2	2	2	2	2	2
11	2	2	2	2	2	2	2	2	2	3	2	2
12	1	1	1	1	1	1	2	3	3	2	2	2
13	2	1	2	1	2	1	3	3	3	3	3	3
14	1	1	1	1	1	1	3	3	3	3	3	3
15	2	1	1	2	2	2	3	2	3	3	2	2
16	2	2	2	3	2	2	3	3	1	3	3	3
17	1	1	1	1	1	1	3	3	3	3	3	3
18	3	1	1	1	1	1	3	3	3	2	2	2
19	2	2	2	2	2	2	3	3	3	3	3	3
20	2	2	2	2	2	2	2	2	2	2	2	2

SUMMARY OF DATA

ENAMEL MARGINS

GRADES	CONTROL GROUP	FRIST EXPERIMENTAL GROUP	SECOND EXPERIMENTAL GROUP
GRADE 0	26	22	0
GRADE 1	72	60	57
GRADE 2	16	36	57
GRADE 3	6	2	6
TOTAL	120	120	120

DENTIN MARGINS

GRADES	CONTROL GROUP	FRIST EXPERIMENTAL GROUP	SECOND EXPERIMENTAL GROUP
GRADE 0	0	0	0
GRADE 1	13	0	13
GRADE 2	29	43	50
GRADE 3	78	77	57
TOTAL	120	120	120

APPENDIX 3
MICROLEAKAGE

Descriptives

			Statistic	Std. Error
Enamel margins control group	Mean		.9250	.13705
	95% Confidence Interval for Mean	Lower Bound	.6381	
		Upper Bound	1.2119	
	5% Trimmed Mean		.8889	
	Median		1.0000	
	Variance		.376	
	Std. Deviation		.61291	
	Minimum		.00	
	Maximum		2.50	
	Range		2.50	
	Interquartile Range		.00	
	Skewness		.505	.512
	Kurtosis		1.794	.992

			Statistic	Std. Error
Enamel margins first experimental group	Mean		1.1250	.12500
	95% Confidence Interval for Mean	Lower Bound	.8634	
		Upper Bound	1.3866	
	5% Trimmed Mean		1.1389	
	Median		1.0000	
	Variance		.313	
	Std. Deviation		.55902	
	Minimum		.00	
	Maximum		2.00	
	Range		2.00	
	Interquartile Range		.50	
	Skewness		.204	.512
	Kurtosis		-.250	.992

			Statistic	Std. Error
Enamel margins second experimental group	Mean		1.6000	.11239
	95% Confidence Interval for Mean	Lower Bound	1.3648	
		Upper Bound	1.8352	
	5% Trimmed Mean		1.5833	
	Median		1.7500	
	Variance		.253	
	Std. Deviation		.50262	
	Minimum		1.00	
	Maximum		2.50	
	Range		1.50	
	Interquartile Range		1.00	
	Skewness		-.097	.512
	Kurtosis		-1.509	.992

			Statistic	Std. Error
Dentin margins control group	Mean		2.5250	.14269
	95% Confidence Interval for Mean	Lower Bound	2.2263	
		Upper Bound	2.8237	
	5% Trimmed Mean		2.5833	
	Median		3.0000	
	Variance		.407	
	Std. Deviation		.63815	
	Minimum		1.00	
	Maximum		3.00	
	Range		2.00	
	Interquartile Range		1.00	
	Skewness		-.946	.512
	Kurtosis		-.238	.992

			Statistic	Std. Error
Dentin margins first experimental group	Mean		2.7000	.10513
	95% Confidence Interval for Mean	Lower Bound	2.4800	
		Upper Bound	2.9200	
	5% Trimmed Mean		2.7222	
	Median		3.0000	
	Variance		.221	
	Std. Deviation		.47016	
	Minimum		2.00	
	Maximum		3.00	
	Range		1.00	
	Interquartile Range		1.00	
	Skewness		-.945	.512
	Kurtosis		-1.242	.992

			Statistic	Std. Error
Dentin margins second experimental group	Mean		2.3500	.14555
	95% Confidence Interval for Mean	Lower Bound	2.0454	
		Upper Bound	2.6546	
	5% Trimmed Mean		2.3889	
	Median		2.2500	
	Variance		.424	
	Std. Deviation		.65091	
	Minimum		1.00	
	Maximum		3.00	
	Range		2.00	
	Interquartile Range		1.00	
	Skewness		-.649	.512
	Kurtosis		-.242	.992

NPar Tests
Kruskal-Wallis Test

Ranks

	Group	N	Mean Rank
Enamel margins groups	1	20	22.88
	2	20	27.60
	3	20	40.03
	Total	60	
Dentin margins groups	1	20	30.00
	2	20	33.95
	3	20	25.84
	Total	60	

Test Statistics[a,b]

	Enamel margins groups	Dentin margins groups
Chi-Square	12.225	2.801
Df	2	2
Asymp. Sig.	.002	.247
	Statistically significant differences	Differences not Statistically significant
a. Kruskal Wallis Test		
b. Grouping Variable: Group		

Mann-Whitney Test

Ranks

	Group	N	Mean Rank	Sum of Ranks
Enamel margins groups	1	20	18.80	376.00
	2	20	22.20	444.00
	Total	40		

Test Statistics[b]

	Enamel margins groups
Mann-Whitney U	166.000
Wilcoxon W	376.000
Z	-1.041
Asymp. Sig. (2-tailed)	.298
Exact Sig. [2*(1-tailed Sig.)]	.369[a]

a. Not corrected for ties.

b. Grouping Variable: Group

Mann-Whitney Test

Ranks

	Group	N	Mean Rank	Sum of Ranks
Enamel margins groups	1	20	14.58	291.50
	3	20	25.71	488.50
	Total	40		

Test Statistics[b]

	Enamel margins groups
Mann-Whitney U	81.500
Wilcoxon W	291.500
Z	-3.345
Asymp. Sig. (2-tailed)	.001
Exact Sig. [2*(1-tailed Sig.)]	.002[a]

a. Not corrected for ties.

b. Grouping Variable: Group

Mann-Whitney Test

Ranks

	Group	N	Mean Rank	Sum of Ranks
Enamel margins groups	2	20	15.90	318.00
	3	20	24.32	462.00
	Total	40		

Test Statistics[b]

	Enamel margins groups
Mann-Whitney U	108.000
Wilcoxon W	318.000
Z	-2.448
Asymp. Sig. (2-tailed)	.014
Exact Sig. [2*(1-tailed Sig.)]	.021[a]

a. Not corrected for ties.

b. Grouping Variable: Group

Wilcoxon Signed Ranks Test

Ranks

		N	Mean Rank	Sum of Ranks
MDMC – MEMC	Negative Ranks	1[a]	1.50	1.50
	Positive Ranks	18[b]	10.47	188.50
	Ties	1[c]		
	Total	20		

a. MDMC < MEMC

b. MDMC > MEMC

c. MDMC = MEMC

Test Statistics[b]

	MDMC - MEMC
Z	-3.819[a]
Asymp. Sig. (2-tailed)	.000

a. Based on negative ranks.

b. Wilcoxon Signed Ranks Test

Wilcoxon Signed Ranks Test

Ranks

		N	Mean Rank	Sum of Ranks
MDME1 - MEME1	Negative Ranks	0[a]	.00	.00
	Positive Ranks	19[b]	10.00	190.00
	Ties	1[c]		
	Total	20		

a. MDME1 < MEME1

b. MDME1 > MEME1

c. MDME1 = MEME1

Test Statistics[b]

	MDME1 - MEME1
Z	-3.866[a]
Asymp. Sig. (2-tailed)	.000

a. Based on negative ranks.

b. Wilcoxon Signed Ranks Test

Wilcoxon Signed Ranks Test

Ranks

		N	Mean Rank	Sum of Ranks
MDME2 - MEME2	Negative Ranks	2[a]	8.75	17.50
	Positive Ranks	14[b]	8.46	118.50
	Ties	4[c]		
	Total	20		

a. MDME2 < MEME2

b. MDME2 > MEME2

c. MDME2 = MEME2

Test Statistics[b]

	MDME2 - MEME2
Z	-2.658[a]
Asymp. Sig. (2-tailed)	.008

a. Based on negative ranks.

b. Wilcoxon Signed Ranks Test

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