MICROLEAKAGE IN CLASS II COMPOSITE RESTORATIONS BONDED WITH DIFFERENT ADHESIVE SYSTEMS

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By

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DEDICATION

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

To my wife and children who **spread joy and happiness in my life***.*

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

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ABSTRAK

Objektif: Tujuan kajian ini ialah untuk membandingkan keberkesanan dua sistem perlekatan iaitu sistem perlekatan “self-etch”, “one-step”, “one-component” dan sistem perlekatan etsa dan bilas. Kajian ini juga digunakan untuk membandingkan dua jenis bahan iaitu nanokomposit dan mikrohibrid komposit ke atas kebocoran mikro pada tampalan komposit kelas II dalam dentin. Keberkesanan penggabungan sistem perlekatan “self-etch”, “one-step”, “one-component” dengan nanokomposit keatas kebocoran mikro pada tampalan komposit kelas II dalam dentin juga dikaji. *Bahan bahan dan langkah-langkah:* Lima puluh dua batang gigi premolar kekal bahagian atas telah digunakan dan 2 lubang kelas II (3 millimeter (mm) lebar x 1.5 mm dalam) dengan pinggir gingiva berada 1 mm di bawah CEJ telah disediakan dan tampalan dilakukan pada setiap gigi. Dua sistem perlekatan “self-etch”,”one-step”,”one-component” (G Bond, GC, Japan), sistem perlekatan etsa dan bilas (Adper Single Bond 2, 3M ESPE, USA) dan dua bahan komposit: nanokomposit (Filtek Z350, 3M ESPE, USA) dan mikrohibrid komposit (Filtek Z250, 3M ESPE, USA) telah digunakan dalam kajian ini mengikut arahan daripada pihak pengeluar. Seratus empat kaviti tersebut dibahagi secara rawak kepada 4 kumpulan (n=26). Dua kumpulan yang pertama telah ditampal menggunakan Filtek Z350 (3M ESPE, USA) manakala dua kumpulan terakhir ditampal menggunakan Filtek Z250 (3M ESPE, USA). Selain itu, semua kaviti daripada kumpulan 1 dan 3 dilekatkan menggunakan “G Bond” (GC, Japan) manakala kumpulan 2 dan 4 dilekatkan menggunakan “Adper Single Bond 2” (3M ESPE, USA). Spesimen ditermosikal pada suhu 5˚- 55˚C selama 30 saat sebanyak 500 kitaran. Sampel kemudian direndam di dalam 0.5% dakwat Rhodamine B selama 10 jam dan dipotong secara longitudinal. Penetrasi dakwat pada pinggir gingiva diukur dalam millimeter menggunakan “confocal laser scanning microscope” (CLSM) pada 10x magnifikasi. Data dianalisa menggunakan “Two-Way ANOVA” dan keputusan dengan $p<0.05$ dianggap signifikan secara statistik. *Keputusan:* Tiada perbezaan signifikan dijumpai ($p>0.05$) untuk sistem perlekatan “self-etch”, “one-step”, “one-component” (G Bond) dan sistem perlekatan etsa dan bilas (Adper Single Bond 2). Penetrasi dakwat antara bahan komposit yang digunakan juga menunjukkan tiada perbezaan signifikan ($p>0.05$). Tiada perbezaan yang signifikan dapat dilihat dalam penetrasi dakwat pada pinggir gingiva dalam semua kumpulan kajian. *Kesimpulan:* Sistem perlekatan “self-etch”, “one-step”, “one-component” dan bahan tampalan nanokomposit memberi keputusan yang sama dengan sistem perlekatan etsa dan bilas dan mikrohibrid komposit dalam mengurangkan kebocoran mikro pada tampalan komposit kelas II.

MICROLEAKAGE IN CLASS II COMPOSITE RESTORATIONS BONDED WITH DIFFERENT ADHESIVE SYSTEMS

ABSTRACT

Objectives: The purpose of this study was to compare the effect of self-etch, one-step, one-component adhesive system and etch and rinse adhesive system as well as the nanocomposite and microhybrid composite on the microleakage of class II composite restorations located in dentin. The effect of the combination of self-etch, one-step, one-component adhesive and nanocomposite on the microleakage of class II composite restorations located in dentin was also investigated. *Materials and Methods*: Fifty two upper permanent premolar teeth were used and two class II cavities (3 millimeter (mm) width x 1.5 mm depth) with gingival margins ended 1mm below CEJ were prepared and filled in each tooth. Two adhesive systems: self-etch, one-step, one-component adhesive system (G Bond, GC, Japan), etch and rinse adhesive system (Adper Single Bond 2, 3M ESPE, USA) and two composite materials: nanocomposite (Filtek Z350, 3M ESPE, USA), microhybrid composite (Filtek Z250, 3M ESPE, USA) were used and applied in this study according to the manufacturers instructions. The 104 cavities were divided randomly into four groups (n=26). The first two groups were restored with Filtek Z350 (3M ESPE, USA) while the last two groups were restored with Filtek Z250 (3M ESPE, USA). All the cavities in group 1 and 3 were bonded with G Bond (GC, Japan) while the cavities in group 2 and 4 were bonded with Adper Single Bond 2 (3M ESPE, USA). The specimens were thermocycled between 5° to 55° C with 30 second dwell time for 500 cycles. The samples were then immersed in 0.5% Rhodamine B dye for 10 hours and sectioned longitudinally. Dye penetration at the gingival margin was quantified in millimeters under confocal laser scanning microscopy (CLSM) at 10x magnification. Data were analyzed using Two-Way ANOVA and results with $p<0.05$ were considered statistically significant. *Results*: No significant difference (*p*>0.05) in dye penetration was discovered between self-etch, one-step, one-component adhesive system (G Bond) and etch and rinse adhesive system (Adper Single Bond 2). No significant difference (*p*>0.05) was also found in dye penetration between the composite materials used. No significant difference (*p*>0.05) in dye penetration was observed on the gingival margin among all study groups. *Conclusion*: Self-etch, one-step, one-component adhesive system and nanocomposite restorative materials produced similar results to those of etch and rinse adhesive systems and microhybrid composites in microleakage of class II composite restorations.

CHAPTER ONE

INTRODUCTION

1.1 Background of the Study

Good adhesion between dentin and restorative resins is of primary importance in clinical practice. The search for improved adhesive and resin composite materials has received considerable interest in the recent years. The improved properties of adhesive materials, resin-based composite restorations have been made more reliable and long-standing .

New approaches to bonding restorative resins to tooth substrates without acid etching step, such as self-etching systems, have recently been introduced. These simplified systems aim to reduce technique sensitivity by reducing the number of clinical steps involved (Van Meerbeek *et al.*, 2003). As a result, their popularity is increasing.

One of the advantages of using single-step, self-etch, one-component adhesives is, it can prevent discrepancies occurring between the depth of etching and resin monomer penetration. This is because the single-step, self-etch, one-component adhesive systems form a continuous layer by simultaneous demineralization with acidic monomers, followed by resin monomer penetration into the dentin substrate (Van Meerbeek *et al.*, 2003).

Unfortunately, the first evaluations of the sixth (6th) generation system showed sufficient bond to conditioned dentin but, the bond to enamel was less effective (Kugel and Ferrari, 2000). This may be due to insufficient etching to enamel. In addition, they are available in two bottles by which one drop of liquid from each will be mixed together before application to the tooth structure. This can cause error to occur due to unequal ratio of liquid mixed or mishandling of the bottles. As a result, some manufacturers introduced the 7th generation one bottle adhesive systems which simplifies the procedure further.

Although there have been reports regarding the performance of total-etch and self-etch (5th and 6th generations) adhesive systems, studies and reported data on the capability of the newly introduced self-etch, one-step, one-component adhesive system (7th generation) in sealing the margins of restorations in class II cavities is very limited. Moreover, most of the previously conducted studies on self etch adhesives tested these materials on Class V preparations (Deliperi *et al.*, 2007, Brackett *et al.*, 2004, Owens and Johnson, 2007). This is because studies on class V are easy to control and standardize while, studies performed on class II restorations are more difficult to execute and normally have a greater standard deviation. This is due to the significant influences of dentin structures, the operative procedure and the origin of dentin (caries affected, sclerotic, sound, superficial or deep) (Ernst, 2004).

Since the presence of open margins around restorations causes teeth to be susceptible to secondary caries, a restoration should provide a good adhesion to the tooth. Resin shrinkage due to polymerization has been reported as one of the factors associated with marginal leakage and gap formation at the tooth-restoration interface (Cenci *et al.*, 2005). The polymerization shrinkage can create significant stress in the surrounding tooth structure and may lead to bond failure (Amaral *et al.*, 2004). This problem can be minimized by using different restorative techniques and different materials such as nanocomposite. These composites are characterized by using nano particles that increase in the filler loading with the consequences of reducing polymerization shrinkage and increased the mechanical properties (Beun *et al.*, 2007).

Araujo Fde *et al.* (2006) and Fruits *et al.* (2006) reported that major marginal microleakage occurs on the gingival surfaces located in dentin or cementum. Generally, data regarding microleakage of nanocomposites, as well as, nanocomposites bonded with self-etch compared to total etch adhesive systems in class II restorations is still limited. Therefore, it is useful to determine the effect of self-etch, one-step, one-component adhesive materials and nanocomposite at the gingival surface microleakage in class II restorations.

This raises the question whether self-etch, one-step, one-component, adhesive systems and nanofilled composites are efficient in preventing or reducing microleakage in class II cavities and whether the combination of nanofilled composites with the self-etch, one-step, one-component, adhesive systems add an advantage in preventing or reducing microleakage.

Microleakage testing conducted by previous studies (Kim *et al.* 1992, Brunton *et al.* 2004) using normal low-resolution optical microscopy with fluorescent dyes since these dyes present a very visible, strong color. However, with the introduction of confocal laser scanning microscope, microleakage testing starts to rely on the fluorescence criteria of the dye rather than the color, which give us a new advantage over conventional microscopy. Confocal laser scanning microscope offers several other advantages over conventional optical microscopy. This includes the ability to control depth of field, elimination or reduction of background information away from the focal plane (that leads to image degradation) and the capability to collect serial optical sections from thick specimens. This technique generates significant improvement in resolution, lying somewhat between that of conventional light microscope and scanning electron microscope (SEM). Moreover, this type of microscopy enables high-resolution images to be made of samples with minimum requirements for specimen preparation (Watson, 1997). Therefore, CLSM may provide more accurate detection of microleakage (D'Alpino *et al.*, 2006a). For all these reasons, this technology was utilized to detect microleakage in this study.

1.2 Statement of Problem

Due to increase patient’s demand for aesthetic, the use of adhesive restorations for posterior teeth is becoming very popular these days (Mjor and Gordan, 2000). Due to this there is a tendency to simplify bonding procedure to reduce the clinical time and improve the longevity of the restoration. Over time, several changes have been made in formulation to produce materials that enhance clinical success. The latest innovations are the development of dental composites based on nanotechnology. However, problems, such as wear, technique sensitivity and microleakage still the concern when resin-based composite restorations are placed in teeth. Since the nanocomposite materials have been around for only a short period, no independent research data is yet available (Ure and Harris, 2003). Therefore, the aim of this *in vitro* study was to investigate the microleakage in Class II restorations restored with self-etch, one-step adhesives and nanocomposites.

1.3 Justification of Study

Due to increased patients demand for aesthetic restorations and the many availability of products in the market, it is necessary to evaluate the quality and reliability of the new adhesive systems and new nano-composite materials in reducing the microleakage of composite restorations through laboratory investigation.

Since the self-etch, one-step, adhesive systems and nanocomposite materials are used on patients by the students and the clinicians at the dental school of Universiti Sains

Malaysia, it is important to study its efficacy in reducing the microleakage in class II composite restorations.

In addition, the result of the study will provide the dental health service providers with some knowledge that will help them select better treatment modalities in order to provide longer lasting restorations to their patients.

1.4 Objective of Study

1.4.1 General Objectives

The purpose of this *in vitro* experimental study is to evaluate the efficacy of self-etch, one-step, one-component adhesive systems and nanocomposites in reducing the microleakage of class II composite restorations, in relation to the total-etch adhesives and microhybrid composites using confocal laser scanning microscope (CLSM).

1.4.2 Specific Objectives

First objective: To compare the microleakage of self-etch, one-step, one-component adhesive systems (G Bond, GC, Japan) in class II composite restorations with the total-etch adhesive systems (Adper Single Bond 2, 3M ESPE, USA).

Second objective: To compare the microleakage of class II composite restorations restored with nano-composite material (Filtek Z350, 3M ESPE, USA) and microhybrid composite material (Z250, 3M ESPE, USA).

Third objective: To compare the microleakage of the nano-composite material (Filtek Z350, 3M ESPE, USA) bonded with self-etch, one-step, one-component adhesive system (G Bond, GC, Japan) with other study groups (1,2 ,and 3).

1.5 Research Hypothesis

1) Self-etch, one-step, one-component adhesive system has lower microleakage as compared to the total etch system in class II restorations.
2) Nano-composite has lower microleakage as compared to the microhybrid composite material in class II restorations.
3) Nano-composite material bonded to self-etch, one-step, one-component adhesive system exhibit the lowest microleakage as compared to other study groups

CHAPTER TWO

LITERATURE REVIEW

2.1 Microleakage

2.1.1 Introduction and Definition

The integrity and durability of the marginal seal has always been of prime concern in the investigation of dental restorative materials performance. One of the key functions of a dental restoration is to seal the exposed dentin from the oral environment, to prevent pulpal damage and further decay. Therefore, the microleakage at the tooth-restorative interface is a major concern influencing the clinical longevity of composite resin restorations (Gwinnett *et al.*, 1995).

The marginal integrity of the tooth and restoration interface is dependent upon several factors. It is dependent upon type of restorative material, the physical properties of the material, interactions between materials, physical properties of the tissue interface, and the interaction of the oral environment (Rossomando and Wendt, 1995)

The clinical symptoms associated with the occurrence of microleakage are breakdown and discoloration of margins, secondary caries, increase in postoperative sensitivity, pulpal pathology, and reduction in the longevity of the restoration (Peutzfeldt, 2000).

Leakage of water and other products can occur along the interface through voids created during insertion or function. Based upon the size of these voids, two types of leakage can be distinguished:

Microleakage: occurs when large voids are present causing water, large molecules, and even bacteria to migrate along the restoration. Microleakage is defined as the clinically undetectable passage of bacteria, fluids, molecules, or ions between a cavity wall and the restorative material applied to it (Kidd, 1976).

Nanoleakage: occurs when the voids are so small that only water and some small molecules can pass. The difference between both types is somewhat arbitrary, since both may occur simultaneously (Dorfer *et al.*, 2000).

Many different laboratory techniques have been used to demonstrate microleakage. These techniques include the use of bacteria, compressed air, chemical and radioactive tracers, neutron activation analysis, artificial caries technique, scanning electron microscope, and perhaps the most common of all, dye penetration. Investigation of leakage has been carried out both *in vivo* and *in vitro*, but the latter is more common. *In vitro* experiments can be divided into two categories, one that uses a clinical simulation and the other one that is purely a test of the behavior of materials (Kidd, 1976).

2.1.2 Factors Contributing to Microleakage

Several factors can affect the integrity of the tooth-restoration interface and can contribute to microleakage. Among these are:

- Polymerization shrinkage and cavity configuration factor
- Hydroscopic expansion
- Light polymerization concepts and units
- Thermal cycling and occlusal stresses

2.1.2.1 Polymerization Shrinkage and Cavity Configuration Factor

Photo-polymerization is now a widely accepted initiation mode for the clinical hardening processes with a wide range of biomaterials including dental adhesives and restorations. The polymerization of dental composite resins is inevitably accompanied by shrinkage. Due to this, molecular densification during the polymerization process of dental restoratives, and the macroscopic effects of shrinkage strain and/or shrinkage stress, continue to attract widespread international research interest (Watts and Silikas, 2005).

Resin shrinkage due to polymerization has been reported as one of the factors directly responsible for marginal staining, fractures, debonding, microleakage, secondary caries and postoperative sensitivity. The present generation of flowable composites undergoes a free volumetric shrinkage of 4-9% (Watts and Silikas, 2005). For condensable composites, volumetric shrinkage is in the range of 2-6%, with most values near 3.5%. Sixty five to 75% of this shrinkage occurs within the first 10 minutes of placement, irrespective of curing mode. Polymerization stresses in a bonded structure may cause adhesive or cohesive failure and interfacial gap formation or, if adhesion is maintained, deformation of residual tooth structure may occur (Watts and Silikas, 2005).

The effect of tooth structure deformation to accommodate potential stress is unknown. However, light cured composites develop higher stress than auto-cured analogues, and the use of higher energy curing lights further exacerbate the situation. Shrinkage stresses are very dependent upon the cavity geometry as well as the underlying chemistry. This is often discussed in terms of C-factor. C-factor is defined as the ratio of the bonded surface area to the free unbonded surface area of the cavity (Albers, 2002).

C-factors for dental restorations typically range from 0.1 to 5.0 with higher values (>1.5) indicating more likelihood of higher interfacial stresses. A key effect on actual stress is dependent on the complexity of a dental restoration. For example, Class I and deep Class V cavities have high C-factor values with possible high contraction stresses. However, the presence of a high C-factor is a risk for bonding because the polymerization stresses may be too great to be counteracted by the bond strength of the dentine bonding agent. Immediate bond strength of approximately 17 MPa may be necessary to resist the contraction stresses to prevent marginal debonding (Bayne *et al.*, 2002).

Because of its simplicity, the C-factor theory has been an attractive explanation for potential clinical problems. However, the real importance of these effects for current clinical systems may be much less. Many composites are highly filled, placed incrementally, and cured with appropriate techniques. In addition, newer adhesive systems are much better bonded (Bayne *et al.*, 2002).

Braga *et al.* (2006) demonstrated that shrinkage stress and microleakage were higher in restorations with larger diameters and depths. In addition, they stated that high stresses may be translated into a more severe microleakage or low bond strength, depending on the variable tested. However, microleakage seemed to be related to restorations volume, but not to its C factor. Cara *et al.* (2007) examined the effect of polymerization contraction stress (manifested as cuspal deflection) during polymerization of the resin based composites. They demonstrated that a precured intermediary flowable layer reduced the associated cuspal deflection resulting from the polymerization shrinkage of the incrementally applied resin based composites. Nevertheless, this precured intermediary flowable layer did not affect the microleakage.

2.1.2.2 Hydroscopic Expansion

Polymerization reaction and subsequent interaction with the aqueous oral environment may result in a series of physical changes in the resin-based composite restorative materials. Resin-based composite restorative materials may absorb significant amounts of water when exposed to the oral environment. Water sorption may produce some undesirable effects such as dissolution, hydrolysis, expansion, plasticization, microcrack formation and fatigue. This degradation of its physical properties decreases the life expectancy of dental restorative materials (Robenson *et al.*, 2002).

Most polymer-based materials absorb water through a diffusion controlled process. A number of factors including type of resin, filler fraction, filler size, reactivity of the glass and the presence of silane and non-silane coupling agents, determine the diffusion coefficient of dental composite restorative materials (Eliades *et al.*, 2005). Of these, the nature of the resin matrix has the most significant bearing on the amount and rate of hydroscopic expansion for any given resin-based composite restorative material. Water sorption by the resin may compensate for the effect of the polymerization shrinkage. This is because; the resin may expand and seal off marginal gaps. However, it occurs only over a relatively long period of time and it is directly proportional to resin content (Perdigao and Swift, 2002; Robenson *et al.*, 2002).

Unfortunately, swelling is much more marked for restorations with a low C-factor, in which shrinkage stress is not as a great problem. In the case of high C-factor restorations, the surface of the restoration which is exposed to the oral cavity will initially gain in volume. This gain produces a gradient from the outer surface to the bulk of the restoration, thus adding additional stress. On the other hand, owing to the slow process of water sorption from saliva, stress relief may come too late, after fractures have already formed. Although water sorption is generally recognized as a stress-relieving mechanism, there are only a few quantitative data available to assess its true impact. After a prolonged period of swelling, nonshrinking composite materials may encounter major problems related to expansion stress in some types of restorations for example, in mesioocclusodistal (MOD) restorations (Dauvillier *et al.*, 2000).

2.1.2.3 Light Polymerization Concepts and Units

In recent years, several new polymerization techniques and curing units have been introduced in an attempt to affect polymerization shrinkage and to reduce the resin curing time. Conventional quartz halogen curing lights with higher intensities, plasma arc curing lights, blue light emitting diode curing lights (LED), and argon lasers are predominated in clinical practice for polymerization of direct resin-based restorative materials (Albers, 2002).

According to Albers (2002), two categories of techniques are commonly used in curing the resin composites, which is *Continuous* and *Discontinuous technique.*

Continuous curing technique: It refers to a light cure sequence in which the light is exposed continuously. There are four types of continuous curing:

Uniform continuous cure: Where a light of a constant intensity is applied to a composite for a specific period of time. This is the most familiar method of curing currently in use.

Step cure: The composite cured first at low energy, then stepped up to high energy.

Ramp cure: The light is initially applied at low intensity and gradually increased over time to high intensity. It attempt to pass through all the different intensities in hopes of optimizing polymerization.

High-energy pulse: It uses a short pulse (10 seconds) of extremely high energy (1000-2800 mW per cm^2). However, most of these techniques are conducted with halogen, arc, and laser lamps (Albers, 2002).

Discontinuous curing technique: It is also called soft cure, which commonly uses a pulse delay mode of curing. It is similar to holding a halogen light at some distance from a tooth to initiate a cure, and then moving it close to the restoration for the duration of appropriate exposure. Soft start settings are available on some halogen curing lights (Albers, 2002).

However, halogen lamps have the flexibility to apply energy at a range from low to high and for various lengths of time. Although appealing in concept, arc and laser units invariably apply large amounts of light and therefore appropriate for continuous or pulse curing. This limits their flexibility for clinical applications. The blue light-emitting diode lights (LED) are ideal for battery-powered curing units that are not used for extended time (Albers, 2002).

Amaral *et al.* (2005) evaluated the marginal microleakage of class II resin composite restorations polymerized with blue light-emitting diode units (LED), with comparison to the conventional halogen lamp. They found that the LED light curing units present similar results in controlling the microleakage when compared to conventional halogen lamps. Meanwhile, Cenci *et al.* (2005) found that the polymerization technique had no influence on the microleakage and bond strength of class II composite restorations.

2.1.2.4 Thermocycling

It is noted that there is no concrete evidence that failures in practice occur because of thermal stresses. However, *in vitro* exposure of extracted teeth restorations to cyclic thermal fluctuations has been common in many tracers penetration, marginal gap and bond strength laboratory tests. This is to simulate one of many factors that occur in the oral environment. Although temperature, temperature tolerance, number of cycles and testing medium is still controversy, the laboratory simulations of clinical service are often performed because clinical trials are costly and time consuming (Gale and Darvell, 1999).

Thermal stresses can be pathogenic in two ways. Firstly, mechanical stresses induced by differential thermal changes can induce crack propagation through bonded interfaces. Secondly, the changing gap dimensions are associated with gap volume changes, which pump pathogenic oral fluids in and out of the gaps. However, when materials create an initially intact seal, the interaction of the laboratory test is mainly to predict clinical service. If the intention of the test is not to determine material serviceability but to investigate the mode of failure, then thermal cycling might be appropriate (Gale and Darvell, 1999).

Rossomando and Wendt (1995) investigated the thermocycling and dwell times in microleakage evaluation of bonded restorations. They stated that although a simple review of the literature in the last few years would tend to support limited or no effects of the thermal insult for composite restorations thermocycled with short dwell times, evaluation of microleakage must include thermocycling in order to simulate intraoral conditions. However, the relationship between thermal expansion and the duration of the temperature exposure is an important factor in evaluating the microleakage potential of a restorative material. While unfilled resins and resin composite restorative materials have relatively higher linear coefficients of thermal expansion as compared to the tooth structures, they are extremely good thermal insulators. This insulating characteristic complicates the influence of thermal expansion. However, they suggested that ten seconds (10 s) dwell time is more clinically relevant.

Dorfer *et al.* (2000) investigated the influence of different dentin bonding agents, thermocycling and etching time on nanoleakage phenomenon. They found that thermocycling had no statistical influence on nanoleakage. In addition, Kubo *et al.* (2004) stated that cervical resin composite restorations tended to display more microleakage when they were subjected to thermocycling, but the differences were not statistically significant.

Wahab *et al.* (2003) found that thermocycling significantly increased the microleakage of Class V composite restorations. However, Gale and Darvell (1999) reported that thermal stressing of the restoration interface is only of value when the initial bond is already known to be reliable.

2.1.3 Microleakage Test Methods and Dyes

2.1.3.1 Methods of Microleakage Detection

Diversity in *in vitro* microleakage methods have been used to demonstrate and assess microleakage between tooth and restorative material. According to Van Meerbeek *et al.* (2003), methods of assessing microleakage can be divided into qualititative, semiquantitaive or true quantitative measurements of sealing effectiveness.

i- Qualitative Measurements of Sealing Effectiveness

This method involves immersion of a restored tooth into a dye solution after having the unrestored tooth parts covered with a waterproof varnish close to the restoration margins. After a certain time interval, the specimens are washed and sectioned into two or more slices, to visually determine the extent of a dye penetration along the restoration margins.

ii- Semiquantitaive Measurements of Sealing Effectiveness

This method assumes that if the forces generated during shrinkage or thermo-mechanical strains exceed the bond strength to enamel or dentin, an observable gap will form at the margin of the restoration. These gaps are evaluated by scanning electron microscope (SEM).

iii- Quantitative Measurements of Sealing Effectiveness or Flow Measurements

In this method, the adhesively restored tooth is brought under pressure with water from inside the dental pulp. The permeability or the water flow along the tooth restoration interface is then quantitatively determined through accurate measurement of the displacement of an air bubble within a water filled micropipette, using a computer-driven optical system.

de Almeida *et al.* (2003) studied three different methods to evaluate microleakage of packable composites in class II restorations. The results showed that Rhodamine B detected more microleakage than calcium (Ca) or methylene blue. These results were supported by Amarante de Camargo *et al.* (2006), who reported that the results of dye penetration suggested that there was a difference between dyes and measurement methods, and this should change the interpretation of microleakage tests.

2.1.3.2 Fluorescent Dye

Different techniques using different dye solutions were reported to study microleakage in the literature. Dyes used in dental research are provided either as solutions or particle suspensions of differing particle size, depending upon manufacturer and individual behavior of the dye.

Fluorescent dye is one of the dyes used for microleakage test. It is a very powerful investigative technique in microscopy (Watson, 1997). It is incorporated into adhesive system components, placed in pulp chamber and allowed to diffuse toward the restorative interface, as well as being used as visible tracer in microleakage test (D'Alpino *et al.*, 2006c). Furthermore, fluorescent dyes are useful as tracers because they are detectable in dilute concentrations, inexpensive, and non-toxic, allowing use in clinical as well as laboratory investigations (D'Alpino *et al.*, 2006c).

Fluorescent dyes were used by many researchers. Mohan and Kandaswamy (2005) used Rhodamine B dye in investigation and evaluation of the resin-dentin interface. Likewise, de Almeida *et al.* (2003) used Rhodamine B dye for microleakage evaluation of packable composites in Class II restorations. In the mean time, D'Alpino *et al.* (2006b) found that tow-photon laser microscopy which used fluorescent dyes as its investigative tool provides high quality, high resolution images and precise assessment to the tooth-restoration interface.

Although Watson, (1997) stated that microleakage studies performed with fluorescent dyes and examined using confocal microscopy may provide a more accurate description of restorative failure, most studies that used fluorescing agents did not use confocal microscopy to test for microleakage. Instead, normal, low-resolution optical microscopy was used. For this type of study, the dyes such as Rhodamine B red dye are used because they present a very visible, strong color, and not because of their fluorescence (D'Alpino *et al.*, 2006a)

However, it must be remembered not to overload the materials with dye as it can lead to quenching (an anomalous reduction in fluorescence intensity), and thus cause misleading results. In such conditions, the dye molecules may be located too close to one another. As a consequence, photons emitted from a dye molecule, following interaction with the excitation illumination are absorbed by closely neighbouring molecules rather than emitted as fluorescent light. Therefore, dyes that can bind to tooth substance or to the restorative materials are a potential source of error in leakage studies because penetration studies in dentine also exhibit some dentine staining that should be distinguished from the actual gap between the cavity wall and the restorative material. As such, material properties should be checked to ensure that the dye is not changing the characteristics of the substances under investigation (Watson, 1997).

2.2 Adhesive System

2.2.1 Introduction and Definition

Adhesion describes the attachments of one substance to another whenever they come into close contact with each other. It can be defined as the force that binds dissimilar materials together when they are brought into intimate contact (Blunck, 2000). In order to obtain a better contact between the two materials, an intermediate layer, called an adhesive has to be placed. The two main theories for the observed phenomena of adhesion are mechanical theory and adsorption theory. Mechanical theory states that solidified adhesive, interlocks micromechanically with the roughness and irregularities of the adherends surface. On the other hand, the adsorption theory refers to all kinds of chemical bonds between the adhesive and the adherend, including primary and secondary valence forces. However, the intimate contact of materials depends on the wettability of the substrate, the viscosity of the adhesive, morphology and roughness of the substrate (Blunck, 2000).

Research into adhesive systems for attachment of resins to tooth structure was initiated in early 1950s. The first attempt to develop an adhesive system for bonding acrylic resins to tooth structure was made in 1949 by Hagger. He was a Swiss chemist who invents the first commercially known adhesive system, Sevriton system. The system was based on glycerophosphoric acid dimethacrylate. It was proved to be successful in bonding acrylic resin to the walls and floor of the tooth by forming an intermediate layer which is now called the hybrid zone (McLean, 2000).

In 1955, Buonocore conducted experiment on enamel surface employing a 30-second treatment of 85% phosphoric acid to achieve a simple acid decalcification. He showed that there was a tremendous increase in surface area due to the acid etching action, exposing the organic framework of enamel. Droplets of self-curing resin were attached to the etched area and he demonstrated that resin droplets on treated teeth remained intact for an average of 1,070 hours as compared to 12.2 hour on the untreated enamel surfaces.

In the late 1960s, Buonocore *et al.* (1968) suggested that it was the formation of resin tags that caused the principal adhesion of the resins to acid-etched enamel. However, it took the clinician long time to accept acid etching as part of the treatment. However, the idea that resin penetrates the microporosities of etched enamel and results in a micromechanical bond is well accepted today.

Since then, many improvements have occurred in the formulation and technique of etching enamel and dentin, adhesives, composites and techniques which revolutionized adhesive dentistry. Recently, an increase in the popularity of simplified self-etch adhesive systems and the extensive search for a new adhesive systems which are less technique sensitive, user-friendly and simpler lead to the invention of self-etch, one-step, one-component adhesive system. In this adhesive system, the etching, priming and bonding solutions are combined and mixed in one bottle to become one solution. Based on this combination, this adhesive system can accomplish etching, priming and bonding simultaneously to enamel and dentin immediately after dispensing (Tay and Pashley, 2005).

Due to the chewing forces a composite restoration needs to withstand, effective bonding to tooth structure is of primordial importance. In addition, a good adhesive should be able to prevent leakage along the restorations margins. Clinically, failure of a composite restoration occurs more often due to inadequate sealing, with subsequent discoloration of the cavity margins, than loss of retention. Nowadays, the main bonding mechanism of current adhesives can be regarded as an exchange process involving substitution of inorganic tooth material by resin monomers that upon in situ setting, become micromechanically interlocked in the created microporosities (Van Landuyt *et al.*, 2005).

2.2.2 Bonding to Enamel

Before the introduction of acid etching of enamel and the use of enamel bonding agents, leakage of oral fluids within the microscopic space between the prepared tooth and restorative materials was a greater concern for resin based composites. However, after Buonocore proved that application of phosphoric acids resulted in an altered surface that increased the bonding of acrylic pills to human teeth, subsequent studies suggested that the formation of resin tags at the interface to acid etched enamel was the leading mechanism of enamel adhesion (Blunck, 2000).

The application of 30%-40% phosphoric acid removes about 10 microns of the superficial enamel, resulting in a rough surface with partly dissolved enamel rods down to a depth of 10-20 microns. The evaluation of morphology of the rods showed different orientations of the crystallite, which are differently attacked by the acids. This resulted in a pattern either with missing peripheries of the rods or missing centers. The etching procedure, which includes sufficient rinsing with water to remove all precipitate, also results in a rough surface area with a dramatically enhanced surface area and extremely high surface energy. This effect causes adhesive wettability to increase and allow the adhesive to get into intimate contact with the conditioned enamel surface, thus forming micromechanical retention to the tooth substrate (Blunck, 2000).

Van Meerbeek *et al.* (2003) describe two types of resin tags interlock within the etched enamel. Macro-tags fill the space surrounding the enamel prisms while numerous micro-tags result from resin infiltration and polymerization within the tiny etch-pits at the core of the etched enamel. The latter are especially thought to contribute the most with regard to the retention to enamel.

However, Tay and Pashley (2005) stated that during the early stages of enamel bonding, few researchers understood that bur-cut tooth surfaces were covered by smear layer. This layer masks the underlying enamel and could not be rinsed off with water. Resins applied to smear layer covered surfaces bonded relatively to weak smear layer, rather than to the underlying hard tissues. Therefore, the bond strength of the early adhesive systems was weak. As smear layers are acid labile, the occurrence of enamel smear layer did not present a challenge in bonding that involves the use of phosphoric acid etching step. Nevertheless, the presence of enamel smear layer may become a potential problem when self-etch adhesives are applied on bur-cut enamel. This is related mainly to the acidity of self-etch adhesive used.

Despite the lack of difference between bond strengths in grounded and intact enamel after phosphoric acid etching, the ultrastructure of the resin-enamel interface in phosphoric acid-etched uncut enamel remains the most variable and by far the most difficult to interpret. This is due to the presence of aprismatic and prismatic etching features along the same interface (Tay and Pashley, 2005).

2.2.3 Bonding to Dentin

Bonding to dentin is more challenging than enamel, because of the complex and hydrated structure of dentin. Dentin is an intricate bonding substrate, and in order to obtain good bonding to dentin, an extended knowledge of the structure and composition of the bonding substrate is indispensable. While enamel makes a uniform bonding substrate that consists of almost 90% inorganic material volume with a very small amount of intrinsic water, dentin is a complex composite material with less than 50% inorganic material and high water content volume (21%). Moreover, the tubular build up of dentin and the resulting outward pulpal water current in vital teeth turn dentin into a complex substrate. In addition, the effect of the tooth type, the bonding location (deep versus superficial dentin, crown versus root dentin), and tooth qualities (young, old or sclerotic dentin) on adhesion must be taken in account (Van Landuyt *et al.*, 2005).

Most commonly, the tooth to which the bond will occur is covered with a smear layer. The smear layer is an adherent layer of debris on tooth surface when they are cut with rotary or hand instruments. The smear layer is revealed by scanning electron microscope (SEM) as a 1-2 micron layer of debris with a mainly granular substructure that entirely covers the dentin. The orifices of dentin tubules are obstructed by debris tags, called smear plugs, which may extend into the tubule to a depth of 1-10 microns. These smear plugs are contiguous with the smear layer (Van Landuyt *et al.*, 2005).

The thickness and morphology of the smear layer probably varies with the method used for producing the smear layer and with the location within dentin in relation to the pulp. But the smear layer is believed to be consisted of shattered and crushed hydroxyaptite, as well as fragmented and denatured collagen. However, early smear layer incorporating non acidic adhesives, applied without prior etching, did not penetrate deeply enough to establish a bond with the intact dentin. Such bonds were prone to cohesive failure of the smear layer. Therefore, two options were established to overcome low bond strengths due to the limited strength of the smear layer. This is through removal of the smear layer (totally or partially) or incorporation by modification of the smear layer in the hybrid layer. However, it has been suggested that adhesive techniques that require smear layer removal are associated with more post operative sensitivity than systems that leave the smear layer in situ (Van Landuyt *et al.*, 2005).

The first adhesives achieving clinically acceptable results were based on the smear layer removal, but recently new smear layer incorporating adhesives have regained its popularity. Although adhesives that rely on smear layer removal used phosphoric acid gel of 30-40 % for conditioning step, alternative etchants with different concentrations such as citric, maleic, nitric oxalic, polyacrylic and pyrovic acids are also used in some adhesives (Van Landuyt *et al.*, 2005). However, a comprehensive knowledge of the structure of dentin and its altered properties should enable the dentist to understand how to achieve satisfactory bonding to dentin.

In regard to adhesive systems that require smear layer removal Van Meerbeek *et al.*, (1998) stated that true chemical adhesion between collagen and methacrylate monomers is unlikely, because of the inert nature of collagen fibrils and the low affinity of the monomers for hydroxyaptite depleted collagen. While for systems that incorporate smear layer in the hybrid layer might have possible chemical interaction with hydroxyaptite crystals available in the smear layer (Van Landuyt *et al.*, 2005). However Inoue *et al.*, 2001 stated that neither the thickness of the hybrid layer nor the length of the resin tags seems to play an important role regarding bond strength.

2.2.4 Wet versus Dry Bonding

When dentin is covered with the smear layer and the dentin tubules are occluded with smear plugs, fluid permeability is almost reduced to zero. After the removal of the smear layer by an acid, dentin permeability through the dentinal tubules increases by more than 90%. It was feared that removal of the smear layer and subsequent wetting of the dentin surface would affect the bond strength between dentin and composite. This is because the dentinal fluid dilutes primer and bonding agents and water contamination of bonding was known to lower the bond strength. However, several adhesive systems has been reported to be able to cope with fluid permeability of dentin after smear layer removal, and a high durable bond can be achieved (Van Landuyt *et al.*, 2005).

Technique sensitivity related to some adhesive systems can in part be attributed to the susceptibility of the collagen scaffold to collapse, thus impeding proper infiltration of the collagen with monomers. Etching dentin removes the mineral phase completely, leaving the collagen fibrils suspended in water. While enamel should preferential be dry to achieve good bonding, a certain amount of water is needed to prevent the collagen fibrils in dentin from shrinking. To overcome this problem and to maintain the structural integrity of interfibrillar spaces in the collagen network, two different approaches can be followed. This is depending on the primer of the adhesive system used. The first approach, known as dry bonding technique which involves air drying of dentin after acid etching, and applying a water based primer which is capable of re-expanding the collapsed collagen meshwork. An alternative approach is to leave dentin moist, thereby preventing any collapse and using an acetone based primers, which is known for its water chasing capacity. This technique is commonly referred to as wet bonding. However, determining how moist the dentin should be to ensure complete water removal by acetone based primer is complicated (Van Landuyt *et al.*, 2005).

Even when collapse of collagen is prevented or when re-expanding occur, incomplete resin infiltration of the hydroxyapatite-depleted collagen can still exist after drying. The nano leakage phenomenon is considered as a manifestation of incomplete resin infiltration of the hybrid layer and of a discrepancy between the depth of demineralization and the depth of resin infiltration (Van Landuyt *et al.*, 2005).

2.2.5 Bond Strength

Several factors affect the quality of the bond including the thickness of the smear layer, water content, variations in resin penetration into the demineralized surface,