Uses of Conventional and Novel Expertise Development to Study Microleakage in Tooth Restoration using Composite Resin

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Semua laporan kemajuan dan laporan akhir yang dikemukakan kepada Bahagian Penyelidikan dan Pembangunan perlu terlebih dahulu disampaikan untuk penelitian dan perakuan Jawatankuasa Penyelidikan di pusat pengajian

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Laporan Akhir Projek Penyelidikan Jangka Pendek

1) **Nama Penyelidik:** Dr. SAM'AN MALIK MASUDI

Nama Penyelidik-Penyelidik Lain *(Jika berkaitan)* :

2) **Pusat Pengajian/Pusat/Unit** : PUSAT PENGAJIAN SAINS PERGIGIAN (PPSG) USM.

3) **Tajuk Projek:** USES OF CONVENTIONAL AND NOVEL EXPERTISE DEVELOPMENT TO STUDY MICROLEAKAGE IN TOOTH RESTORATION USING COMPOSITE RESIN

4) (a) **Penemuan Projek/Abstrak**

*(Perlu disediakan makluman di antara 100 - 200 perkataan di dalam **Bahasa Malaysia dan Bahasa Inggeris** Ini kemudiannya akan dimuatkan ke dalam Laporan Tahunan Bahagian Penyelidikan & Pembangunan sebagai satu cara untuk menyampaikan dapatan projek tuan/puan kepada pihak Universiti).*

"see separate papers"

Abstract

The present invitro study was conducted to evaluate microleakage at the enamel and dentin cavity walls of Class V composite restorations using 'multi-step adhesive technique' and compared with 'single-step adhesive technique'. Twenty freshly extracted human premolar teeth were used and V-shaped cavity design with margins in enamel and dentin are prepared on the buccal surfaces of the premolar teeth, using diamond burs after which they were further divided randomly into two groups. Dimension of the preparation are 4 mm in length, 3 mm in width and 2 mm in depth. The prepared cavities were then restored with Alpha-dent Visible light-cure hybrid-type composites (Dental Technologies, Inc.). For Multi-Step technique, the etchant is Alpha-dent Etching liquid 35% phosphoric acid by weight and Bonding agent (combined with Primer) used in this study is Alpha-dent light cure Enamel/Dentin bonding resin. For Single-Step technique, the etching-bonding liquid used in this study is AQ Bond (SUN Medical Co. Ltd.). After finishing and polishing, teeth were thermo-cycled 800 times; 5-55° C and 20 s. dwell time. The teeth were sectioned longitudinally using double-sided diamond disc with mandrel placed on Amman AF350 cutting machine, to form a dimension of 2 mm thick and 10 mm in diameter. Specimens were then ground and polished on one side with fine grits of silicone carbide paper using Knuth Rotor water cooled polishing machine under 10,000 rpm. The prepared specimens were fixed to SEM *stub* using double-sided adhesive tape and observed on the Scanning Electron Microscope (SEM). The ultra structure was investigated directly using the Scanning Electron Microscope (SEM) without die penetration. Photomicrographs were taken of representative areas (from 0 to 3 in the cavity walls) where area (1) is on enamel of the occlusal wall; area (2) is on dentin and area (3) is on enamel of cervical wall of the cavity at magnifications of 1.000 times for further quantitative marginal

leakage analysis. Five photomicrographs were taken for each specimen. Statistical analysis by STATA and Student t-test was performed and correction for comparisons at p< or = 0.05. It was concluded in this study that Single-Step technique showed no different result in reducing microleakage compared to Multi-Step technique and therefore can be used as an alternative treatment in the clinic. Single-Step technique showed significantly lower leakage compared to Multi-Step technique in dentin, but showed no statistically significant differences in occlusal and cervical enamel.

(b) **Senaraikan Kata Kunci yang digunakan di dalam abstrak:**

Bahasa Malaysia	**Bahasa Inggeris**
	COMPOSITE RESIN, ADHESIVE,
	MICROLEAKAGE

5) **Output Dan Faedah Projek**

(a) Penerbitan *(termasuk laporan/kertas seminar)*
(Sila nyatakan jenis, tajuk, pengarang, tahun terbitan dan di mana telah diterbit/dibentangkan).

1. POSTER PRESENTATION : "THE EFFECT OF CAVO SURFACE DESIGN IN REDUCING MICROLEAKAGE OF POSTERIOR COMPOSITES" ON 25-26 SEPT 2001
BY: Dr. SAM'AN M. MASUDI IN: THE INTERNATIONAL ASSOCIATION FOR DENTAL RESEARCH (IADR) CONFERENCE : KUALA LUMPUR, MALAYSIA.

2. PRESENTATION : "THE EFFECT OF CAVOSURFACE PREPARATION IN REDUCING MICROLEAKAGE OF ANTERIOR COMPOSITE RESTORATION." ON 27 SEPT - 1 OCT 2001
BY: Dr. SAM'AN M. MASUDI IN: THE FEDERATION DENTAIRE INTERNATIONALE (FDI) CONFERENCE : KUALA LUMPUR, MALAYSIA

3. PUBLISHED IN JOURNAL OF MICROSCOPY AND ANALYSIS (IN PROCESS) : "USES OF CONVENTIONAL AND NOVEL EXPERTISE DEVELOPMENT TO STUDY MICROLEAKAGE IN TOOTH RESTORATION USING COMPOSITE RESIN"
BY: Dr. SAM'AN M. MASUDI

(b) **Faedah-Faedah Lain Seperti Perkembangan Produk, Prospek Komersialisasi Dan Pendaftaran Paten :**
(Jika ada dan jika perlu, sila gunakan kertas berasingan)

1. As a basic biomaterial study to develop and formulate new Adhesive material and composite resin material used in Dentistry.
2. To know the characterization of Adhesive and composite resin material used in Dentistry

(c) **Latihan Gunatenaga Manusia**

i) ***Pelajar Siswazah*** Dr. Nik Roslin Suriani Nik Sulaiman

ii) ***Pelajar Prasiswazah:***

iii) ***Lain-Lain :*** Technician, Dental surgery Assistant : Knowledge and improvement in SEM preparation in tooth samples.

6. **Peralatan Yang Telah Dibeli:**

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J/K PENYELIDIKAN
PUSAT PENGAJIAN

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The present invitro study was conducted to evaluate microleakage at the enamel and dentin cavity walls of Class V composite restorations using 'multi-step adhesive technique' and compared with 'single-step adhesive technique'. Twenty freshly extracted human premolar teeth were used and V-shaped cavity design with margins in enamel and dentin are prepared on the buccal surfaces of the premolar teeth, using diamond burs after which they were further divided randomly into two groups. Dimension of the preparation are 4 mm in length, 3 mm in width and 2 mm in depth. The prepared cavities were then restored with Alpha-dent Visible light-cure hybrid-type composites (Dental Technologies, Inc.). For Multi-Step technique, the etchant is Alpha-dent Etching liquid 35% phosphoric acid by weight and Bonding agent (combined with Primer) used in this study is Alpha-dent light cure Enamel/Dentin bonding resin. For Single-Step technique, the etching-bonding liquid used in this study is AQ Bond (SUN Medical Co. Ltd.). After finishing and polishing, teeth were thermo-cycled 800 times; 5-55° C and 20 s. dwell time. The teeth were sectioned longitudinally using double-sided diamond disc with mandrel placed on Amman AF350 cutting machine, to form a dimension of 2 mm thick and 10 mm in diameter. Specimens were then ground and polished on one side with fine grits of silicone carbide paper using Knuth Rotor water cooled polishing machine under 10,000 rpm. The prepared specimens were fixed to SEM *stub* using double-sided adhesive tape and observed on the Scanning Electron Microscope (SEM). The ultra structure was investigated directly using the Scanning Electron Microscope (SEM) without die penetration. Photomicrographs were taken of representative areas (from 0 to 3 in the cavity walls) where area (1) is on enamel of the occlusal wall; area (2) is on dentin and area (3) is on enamel of cervical wall of the cavity at magnifications of 1.000 times for further quantitative marginal

analysis. Five photomicrographs were taken for each specimen. Statistical analysis by STATA and Student t-test was performed and correction for comparisons at p< or = 0.05. It was concluded in this study that Single-Step technique showed no different result in reducing microleakage compared to Multi-Step technique and therefore can be used as an alternative treatment in the clinic. Single-Step technique showed significantly lower leakage compared to Multi-Step technique in dentin, but showed no statistically significant differences in occlusal and cervical enamel.

Introduction

Resin-based materials are widely used as direct restorative materials for establishing the esthetic appearance of anterior and posterior teeth. Resin-based materials known as *Composite* materials, consist of a heterogeneous blend of organic resin and inorganic filler.The introduction of resin based, tooth color restorative materials, with their excellent physical properties, has enabled the dental profession to use mechanical adhesion to enamel by means of the acid etched technique.

The application of *acid etching solution*, frequently 35% to 50% phosphoric acid, etches the enamel and creates a more porous surface. When resin is applied to this surface, tag like projections of resin protrude into the enamel. Mechanical retention of resin to enamel is thus created and forms a *mechanical lock*. Acid etching of enamel also removes debris and *wet* the surface of enamel. Thus, etching provides the possibility of achieving both specific and *mechanical adhesion* between resin and enamel. The adhesion value of composites to etched enamel is between 20 and 24 Mpa. However, the resin matrix of resin based materials are quite viscous and the working time short. These two factors may reduce the ability of the material to wet and flow into the etched regions, partially compromising or negating the effectiveness of the acid etching to decrease marginal leakage.

The placement of low-viscosity *bonding resin* before restorative resin materials has been advocated. Most bonding resin consist basically of the same resin as the matrix used in composite, without the filler and modified so as to posses a high degree of fluidity. The premise is that bonding agent will readily wet and flows into the minute holes and discrepancies created by etching the enamel and then in turn polymerize to the resin matrix. Bonding agent also creates *chemical adhesion* to either enamel or dentine.

Microleakage between filling materials and tooth structures is a major dental problem, which is responsible for cases of secondary/recurrent caries, as well as other tooth inflammation and re-infection. The passage of fluids, bacteria, molecules or ions and even air between a restorative material and a prepared cavity wall of a tooth has been defined as microleakage.

In current technique, the operator applied acid etching; washing; drying and applied bonding resin in separately or called *Multi-Step technique*. Studies of the leakage of saliva around the margins of restorations treated with etching and dentin bonding resins demonstrated some decrease in leakage *in vitro* compared with controls; especially to support functional tensile and shear stresses. Multi-Step technique shows around 50 % successful level of leakage or penetrating margin discoloration (Craig, Robert, 1997).

The new Single-Step technique introduced acid etching liquid, primer and bonding resin in combination that shortened the etching-bonding procedures. It is a simple one-liquid of light-curing dental/ enamel bonding resin for resin-based restorations. It does not require any additional priming or acid etching. It contains 4-META, a high performance adhesive monomer that decalcifies tooth substrate and penetrates through the smear layer to form a hybrid layer creating excellent bonds to dentin and enamel. Recent clinical study claims that 4-META have 90% successful level of *Clinical Evaluation* (Wilder and Swift; 2000; Denehy and Cobb, 2000) and *in vitro* study shows that restoration with 4-META reduced the amount of trans-dentinal antigenic challenge through the tight marginal seal, which may have special implications for this material's capacity to protect the dentin/pulp complex (Kamal, A.M.M. *et al.*; 2000). Other investigation confirms that 4-META adhesive liner can be effectively used as a bonding resin between amalgam restoration and the tooth when it is painted onto the cavity walls prior to amalgam setting. (Abraham *et.al.*; 1999)

1. Definition of Dental composites

A composite material is a product, which consists of at least two distinct phases normally formed by blending together components having different structures and properties. The purpose of this is to produce a material having properties, which could not be achieved from any of the individual components alone. The two main components of *Dental Composite* are the resin phase and the reinforcing filler. The beneficial properties contributed by the resin are the ability to be molded at ambient temperatures coupled with setting by polymerization achieved in a conveniently short time. The beneficial properties contributed by the filler are rigidity, hardness, strength and a lower value for the coefficient of thermal expansion. In addition, if the filler occupies a significant proportion of the volume of a composite material it markedly lowers setting contraction. Three properties: coefficient of thermal expansion, setting contraction and surface hardness, depend almost linearly on the filler content. The effect of filler depends on type, shape, size and amount of filler incorporated and, often, the existence of efficient coupling between the filler and resin.

2. Classification and composition of composite resin

The resins used in composite materials are invariably based in methacrylate monomers, usually dimethacrylates. The most commonly used monomers are Bis GMA (addition product of Bis-Phenol A and glycidylmethacrylate) and urethane dimethacrylate. Together with that is TEGMA (triethylene glycol dimethacrylate, which is a co monomer often, used to control the viscosity of the unmixed materials. Bis GMA consistency is similar to that of thick treacle at room temperature. Blending of filler particle wirh a material of this consistency is difficult and has to use a fluid diluent monomer such as TEGMA to reduce the viscosity. A mixture of three parts Bis GMA and one part TEGMA is typically blended with filler.

The type, concentration, particle size and particle size distribution of the filler used in composite material are major factors controlling properties. Filler commonly used include quartz, fused silica and many types of glass including alluminosilicates and borosilicates, some containing barium oxide.

The first generation of composite materials typically contain 60-80%, by weight, of quartz or glass in the particle size range of 1-50 μm. The particle size may vary within this range. Material containing filler particles of this type are referred to as *conventional composites*.

Another development has been the introduction of a number of products containing submicron particles of silica. These materials are referred to as *microfilled composites* and contain silica particles in the range of 0.01-0.1 μm with a typical mean diameter of 0.04 μm. Only 30-60% filler by weight can be loaded in these materials. New ultra-small filler are being investigated that are from 0.005 to 0.01 μm in diameter and are called nanofillers.

A third series of composite materials contain a blend of both conventional glass or quartz particle together with some submicron, particulate silica. These products are referred to as *hybrid composites.* Using filler loadings about 75% conventional size (1-50 μm) and 8% submicron size (0.04 μm average). These materials enable filler loadings of up to 90% by weight.

Methods of activation: Polymerization may be activated *chemically,* by mixing two components, one of which typically an initiator and the other an activator, or by an external *visible light* source.

For *chemical activation,* many different methods of dispensation are available. The most popular is the "two paste' system. Each paste contains a blend of resin and filler. One paste contains about 1% of a peroxide initiator, such as benzoyl peroxide, whilst the other paste contains about 0.5% of a tertiaryamine activator.

Other systems, which rely on chemical activation, are: Powder/liquid systems; Paste/liquid materials and Encapsulated materials in which two parts of materials are initially separated within the capsule.

Light-activated materials are generally supplied as a single paste, which contains monomers, comonomers, filler and an initiator, which is unstable in the presence of high-intensity visible light. The initiator system comprises a mixture of a diketone and an amine. Camphorquinone is a commonly used diketone, which rapidly forms free radicals in the presence of an amine and radiation of the correct wavelength and intensity.

Conventional, microfilled and hybrid composites are all available as either chemically activated or light-activated products. The ISO Standard for resin-based restorative materials (ISO 4049) classifies materials as being of two types depending upon the intended application.

(1) Type 1 is suitable for the restoration of cavities involving occlusal surfaces (posterior teeth).

(2) Type 2 includes all other polymer-based filling and restorative materials.

The standard further sub-divides materials into three classes as follows:

(1) Class 1 comprises self-curing materials whose setting is activated by mixing an initiator and a activator

(2) Class 2 materials are those whose setting is affected by application of energy from external source such as blue light or heat. These materials are further sub-divided as follows:

- Class 2 group 1 – materials whose use requires the energy to be applied intra-orally
- Class 2 group 2 - materials whose method of use requires the energy to be applied extra-orally. These materials are specifically designed for the production of composite inlays and onlays

(3) Class 3 materials are dual-cure materials, which have a self-curing chemical mechanism but are also cured by the application of external energy.

3. Adhesive of resin-based materials

The development and regular use of adhesive materials has begun to revolutionize many aspects of restorative and preventive dentistry. Attitudes towards cavity preparation are altering since, with adhesive materials, it is no longer necessary to produce large undercuts in order to retain the filling. These techniques are, therefore, responsible for the conservation of large quantities of sound tooth substances, which would otherwise be victim to the dental bur.

Microleakage, a major dental problem which is probably responsible for many cases of secondary caries, maybe reduced or eliminated. New forms of treatment, such as the sealing of pits and fissures on posterior teeth, the coverage of badly stained or deformed teeth in order to improve appearance and the direct bonding of brackets in orthodontics have all grown from the development of adhesive systems.

Adhesion is a process of solid and/or liquid interaction of one material *(adhesive or adherent)* with another *(adherend)* at a single interface. Most instances of *dental adhesion* also are called *dental bonding*. Adhesive bond strength is evaluated by debonding the system. Most situation involving dental adhesion really involved *interphase zone*. The interphase zone is the result of interactions of a layer of intermediate material (*adhesive* or *adherent)* with two surfaces *(adherends)* producing two adhesive interfaces and regarded as having three dimensions. For instance, the *hybrid layer* is and interphase between overlying resin composite, resin adhesive and the underlying mineralized enamel or dentin (Nakabayashi and Pashley, 1998).

The interactions, which occur at the interface, are classified generally in terms of types of atomic interactions, which may be involved. Adhesion is classified as physical, chemical, and/or mechanical bonding. *Physical bonding* involves van der Waals or other electrostatic interactions that are relatively weak. It may be the only type of bonding if surfaces are smooth and chemically dissimilar. *Chemical bonding* involves bond between atoms formed across the interface from the adhesive to the adherend. Because the materials are often dissimilar, the extent to which this bonding is possible is limited and the overall contribution to bond strength is normally quite low. Mechanical bonding is the result of an interface that involves undercuts and other irregularities that produce interlocking of the materials. Almost every cases of dental adhesion is based primarily on mechanical bonding. Chemical bonding may occur as well, but generally makes only a small contribution to the overall bond strength.

3. Acid-etch systems for bonding to enamel

The goal of enamel etching are to clean the enamel, to remove the enamel smear layer, to increase microscopic roughness (Retief et.al, 1986) by removal of prismatic and interprismatic mineral crystals an, and to increase the surface free energy of enamel to produce enough monomer infiltration to seal enamel surface with resin and to contribute to the retention of resin composite restorations.

The surface of enamel is smooth and has a little potential for bonding by micro-mechanical attachment. The common method for producing surface roughness for better mechanical bonding is to grind or etch the surface. Grinding produces gross mechanical roughness but leaves a *smear layer* of hydroxyapatite crystals and denatured collagen that is approximately 1 to 3 μm thick.

Acid etching or conditioning dissolves this layer and produces microscopic relief with undercuts on the surface to create an opportunity for mechanical bonding. If the mechanical roughness produces microscopically interlocked adhesive and adherend with dimensions of less than 10 μm, then the situation is described as *micro-mechanical retention*.

On treatment with certain acids for one minute, the structure of the enamel surface may be modified. A 37% solution or acidified gel of phosphoric acid is the acid of choice for most applications of the acid etches technique. The pattern of etching enamel can vary. The most common (Type 1) involves preferential removal of the enamel prism cores, but the prism peripheries remaining intact. The type 2 etching pattern is the opposite of type 1, involving preferential removal of the peripheries with the cores being left intact. The type 3 etching pattern contains areas, which resemble both type 1 and type 2. The diameter of etched enamel porosity is about 5 μm and this surface now suitable for micromechanical attachment since it contains a myriad of small undercuts into which resins can gain ingress, set and form a *mechanical lock*. Resin from the composite flows into the etched enamel and sets, forming rigid *resin tags*, typically 25 μm long, which retain the filling.

The three major factors which affect the success or failure of acid-etch bonding systems as follows:

(1) The etching time. This should be sufficient to cause effective etching as evidenced by a white, chalky appearance on the treated section of enamel after washing and drying. Etching should not continue long enough for dissolved apatites to reprecipitate as phosphates onto the etched surface. The etching time normally used is between 10 to 60 seconds.

(2) The washing stage. Following etching the enamel surface should be washed with copious amounts of water to remove debris. The washing time usually used is 60 seconds.

(3) The drying stage. The surface of the etched enamel should be very thoroughly dried using oil-free compressed air and maintained in a dry, uncontaminated state prior to application of the resin.

4. Acid conditioning for bonding to dentin

The most significant factors which limits bonding to the dentin, in the absence of any form of dentine pre-treatment, is the presence of the *dentine smear layer*. This layer, which is 3-15 μm thick, prevents interaction of the adhesive with the bulk dentine and this prevents the formation of any effective or durable bond. Any bond, which is formed, is to the surface of the smear layer itself and since this may not be strongly bound to the underlying dentine, the bond strength and sealing ability are compromised. The smear layer is formed by the process of cavity preparation and extends over the whole prepared surface of the dentine and into the dentinal tubules *(smear plug)*. It is loosely bound layer of cutting debris including dentine chips, microorganisms, salivary protein and collagen from the dentine.

It is now recognized that in order to form an effective bond and seal between a restorative resin and dentine the smear layer must be removed, disturbed or modified in some way which allows access to the underlying bulk dentine. The liquids used for dentin pre-treatments prior to bonding are called *conditioners*. They are generally acid solutions, which are capable of dissolving or at least *solubilizing* the smear layer, exposing the underlying dentine to the bonding agent.

It is advantageous if the acid used for dentine conditioning can also be used for acid etching the enamel. Since 37% phosphoric acid has a proven track record as an enamel etchant, its also can be used for dentin conditioner. Rinsing after the conditioning stage is recommended, to remove the smear layer completely, leaving a relatively smooth dentine surface with patent dentinal tubules.

When there is no rinsing stage after conditioning, the smear layer becomes re-deposited on the dentine surface.

5. Priming for dentin bonding

After conditioning and rinsing stage, the next stage is the priming stage. This stage is a key stage in the procedure as it is designed to change the chemical nature of the dentine surface and to overcome the normal repulsion between the hydrophilic dentine and the hydrophobic resin. The priming agents are difunctional materials with a methacrylate group (having affinity for the resin) and another reactive group having affinity for the dentine. The most commonly used primer is hydroxyethylmethacrylate (HEMA). It is the hydrophilic nature of the hydroxyl group, which makes HEMA such an effective priming agent. After priming, the nature of the dentine surface is significantly changed – it being more hydrophobic and ready to accept bonding agent. Some materials are combination of priming and bonding agent.

6. Bonding agent

The bonding agent is normally a fluid resin similar in composition to the products for enamel bonding. It consists of a resin similar to that used in the composite material but contains no filler particles. It is very fluid and readily flows into the etched enamel surface. The bonding agent may be a single component, which is activated by light or may consist of two fluid resins, one containing initiator and the other activator, which require mixing before being applied to the etched enamel. The composite filling material is applied directly to the surface of the bonding agent.

Bonding readily occurs at the unfilled resin to composite interface. This is aided by the fact that surface layers of resins polymerized by a free radical mechanism remain soft and unpolymerized due to the inhibating effect which oxygen has on the polymerization mechanism. On applying a composite material to the surface of a "cured' unfilled resin, mixing of the two resin systems occurs at the interface followed by a degree of co-polymerization and entangling which effectively bonds the filled and unfilled resins together. The resulting shear bond strength achieved between etched enamel and restorative resins is 16-20 Mpa. On dentine, the bonding resin is able to flow over and wet the primed surface to complete the formation of an effective bond.

7. Dentin hybrid Layer

It is believe that efficient dentine conditioning not only involves removal of the smear layer and smear plug but also causes a significant decalcification of intertubular dentine to a depth of a few microns. The decalcification process leaves a three-dimensional collagenous network, which can be infiltrated by primer and resin to form a resin *interpenetration layer* or *hybrid layer*, at the interface between the bulk dentin and the resin (Nakabayashi and Pashley, 1998). Depending on the particular chemistry of a bonding system, the hybrid layer may vary from 1 to 5 μm deep. This hybrid layer can be considered to have a composite structure of two continuous phases, the resin phase and the fibrous collagenous phase, which when the resin is polymerized, strongly binds the resin and dentine together.

The ability of primers and resins (or mixtures of two) to penetrate the demineralized dentine surface is the key to the formation of the hybrid layer. Following demineralization the collagenous network is supported only by moisture and any attempt to rigorously dry the dentine at this stage will lead to collapse of collagen fibers and impair the formation of a hybrid layer. It is recommended that dentine is maintained in a moist state prior to application of the primer in order to prevent collapse of the demineralized collagenous network.

Hybridization is the process of creating a hybrid layer in dental hard tissues. Hybridized dentin is prepared by the diffusion of monomers from the adhesive interface into the demineralized dentin; therefore, the concentration of polymers changes gradually from the interface to the deepest point monomers can reach before their polymerization. Thus, the exact interface between resin and dentin has disappeared and should be regarded as an interphase. Following formation of hybridized dentin and coupling of resin composite to dentinal surface, there is no space for microleakage between materials and dentin; the interphase is diffuse (Nakabayashi and Pashley, 1998).

8. Single-Step technique

Single-Step or *Self-etching* concept leaves smear layer in the place and does not require washing off the tooth surface and seals the dentinal surfaces well. Numerous reports in the 2001 AADR and IADR abstracts support this concept as providing very adequate bonding to dentin and enamel and ensuring postoperative comfort (Christensen, 2001).

A self-etch dental adhesive, 4-META is an adhesive monomer that decalcifies tooth substrate and penetrates through the smear layer to form a hybrid layer and creating bonds to enamel and dentin. This material is a clear homogenous solution and the Base liquid is acid with the pH value is 2.5. After the acid has transformed the smear layer, it is reduced to a residue that is mostly water-soluble.

Application of self-etch dental adhesive will dissolve smear layer and decalcifying the dentine. Once applied to the tooth, however, the acetone evaporates almost immediately. This leaves behind water and monomers, which separate into two phases: water on top and monomers toward the tooth. The monomers penetrate into the tooth and polymerize to form a bonded hybrid layer. The water phase on top contains what remains of the smear layer, and these water drops are removed when the operator gently blows the surface with the air syringe.

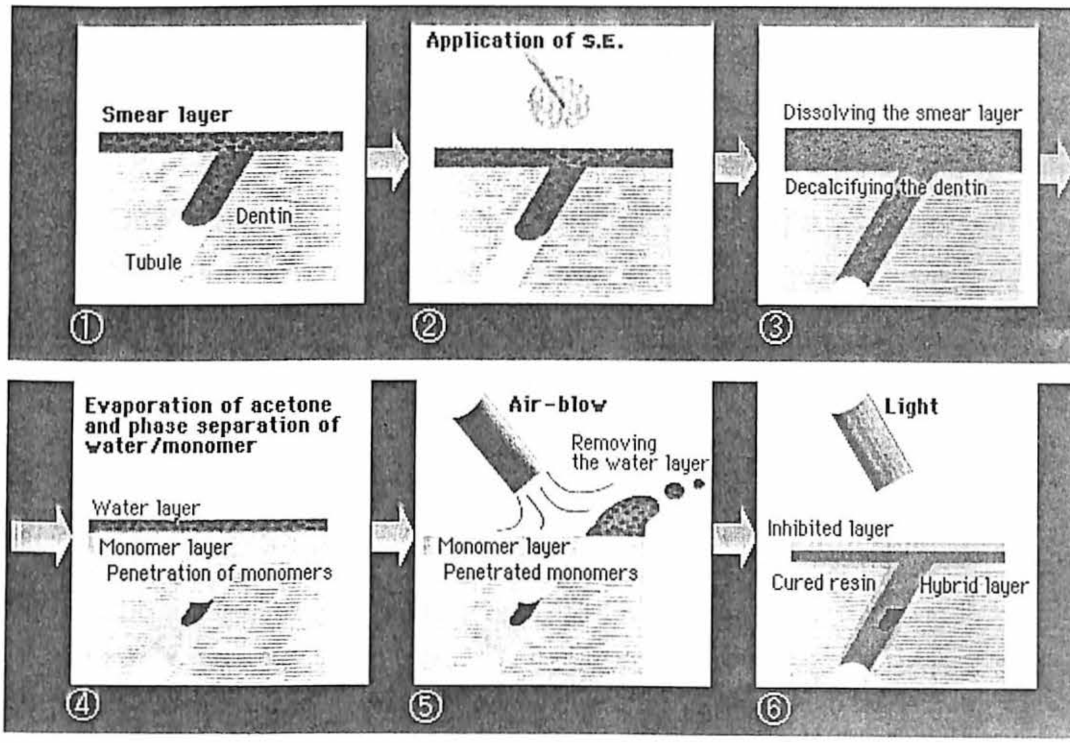

Fig. 1. Application of self-etch dental adhesive on the dentin

This material has been recognized for its high bond strength and minimum leakage (Fitchie *et al.*, 1999; Leinfelder, 1997). The self-etch procedures reduces clinical steps, can be placed inexpensively, provide adequate bonding to dentin and enamel (Christensen, 2001).

9. Conclusion

Multi-Step technique uses three constituents. The first is liquid, usually phosphoric acid, removes the smear layer from the dentin and etches the enamel. The second component, containing hydroxyethylmethacrylate, or HEMA, wetting agent And other ingredients, penetrates the open dentinal canals. The third component usually is unfilled bonding agent, which flows into the open dentinal canals. Correct use of this category of bonding agents forms a hybrid layer of resin-impregnated dentin, forming a seal between the resin-based composite and the open dentinal canals and providing a bond to the dentin.

The main problem with the multi-step technique bonding materials was unpredictable postoperative sensitivity, as reported by dentist as well as patients. The sensitivity probably is related to microleakage and lack of sealing of dentinal canals, because of the very volatile alcohol or acetone containing bonding liquids (Christensen, 2001)

In the One-step technique, all three constituents are placed on the tooth surface at one time and not washed off the tooth surface. Dental practitioners have received this technique very well, and it has captured an impressive increase of number of practitioners. However, before using these bonding systems with restorative resins, dentists should try the respective materials and bonding agents to ensure that the resin restorative materials bonds to another and also tooth structure.

Aim

The purpose of this study is to investigate further, *in vitro,*

- To compare quantitatively the degree of micro leakage in Multi-Step with Single-Step techniques in preventing microleakage around V type class V composite resin restorations, using Scanning Electron Microscope (SEM) investigation.
- To compare the degree of micro leakage in enamel; dentino-enamel-junction and dentin areas of the cavity in Multi-Step with Single-Step techniques in preventing microleakage around V type class V composite resin restorations, using Scanning Electron Microscope (SEM) investigation

Materials and Methods

Twenty erupted human maxillary permanent premolars, extracted for orthodontics treatment purposes, are used in this study. The tooth is collected from the dental clinic. Decision and extraction of the premolars are for orthodontic treatment and done by other dentists/dental specialists (not a Researcher). The teeth were carefully cleaned and then examined at low magnification. No surface showing a development fault or crack or white-spot lesion is used for the experiment.

V-shaped cavity design with margins in enamel and dentin are prepared on the buccal surfaces of the premolar teeth, using diamond burs. Dimension of the preparation are 4 mm in length, 3 mm in width and 2 mm in depth. Samples are divided into 2 (two) groups and randomized using the randomization service of web site : http://www.randomization.com.

The restorative material used in this study is Alpha-dent Visible light-cure hybrid-type composite resin (Dental Technologies, Inc. 6901 North Hamlin Ave., Lincolnwood, Illinois 60712, USA). Alpha-dent composite used in this study is Batch No. LGA-15F; Color B4: 101-003; ISO9001.

For Multi-Step technique, the etchant is Alpha-dent Etching liquid 35% phosphoric acid by weight; Batch No. 271-013. Etchant etches enamel and removes the dentinal smear layer in preparation for bonding. The phosphoric acid liquid etchant has a pH of approximately 0.6 and available in a bottle. Bonding agent (combined with Primer) used in this study is Alpha-dent light cure Enamel/Dentin bonding resin; Batch No. 231-002. Acid-etching liquid (Phosphoric acid 37 %) removes the smear layer from the dentin and etches the enamel. Primer (containing HEMA = hydroxyethyl-methacrylate) wetting resin and penetrates the open dentinal canal. Unfilled bonding resin, which flows into open dentinal canals; forms the hybrid layer of resin-impregnated dentin, forming seal between the composites and open dentinal canals and providing a bond to the dentin and enamel.

For Single-Step technique, the etching-bonding liquid used in this study is AQ Bond (SUN Medical Co. Ltd., 571-2, Furataka-cho, Moriyama, Shiga, Japan) - Batch No. 2003-03.

Table 1. The materials used in this study

No.	Material	Type	Brand	Batch number	Manufacturer
1	Composite resin	Light-cure hybrid	Alpha-dent	LGA-15F	Dental Tech.
2	Etching agent	Liquid 35%	Alpha-dent	271-013	Dental Tech.
3	Bonding agent + primer	Light cure	Alpha-dent	231-002	Dental Tech.
4	Etching-primer-bonding	Liquid	AQ Bond	2003-03	SUN Medical

Restorative material were inserted into two group of cavities in difference techniques:

- First group: Cavities with Multi-Step technique will be treated with acid etching for 15 seconds; the etchant is rinsed off and dried for 15 seconds. The adhesive bonding agent is then applied to the etched enamel and dentin and air dried for 5 seconds and light cured for 10 seconds. Hybrid composite resin filling material is filled into the cavity and light cured.
- Second group: Cavities with Single-Step technique will be treated with etching-bonding liquid in 2 applications. First coat for 20 seconds; drying and second coat for 30 seconds; drying and light cured for 10 seconds.

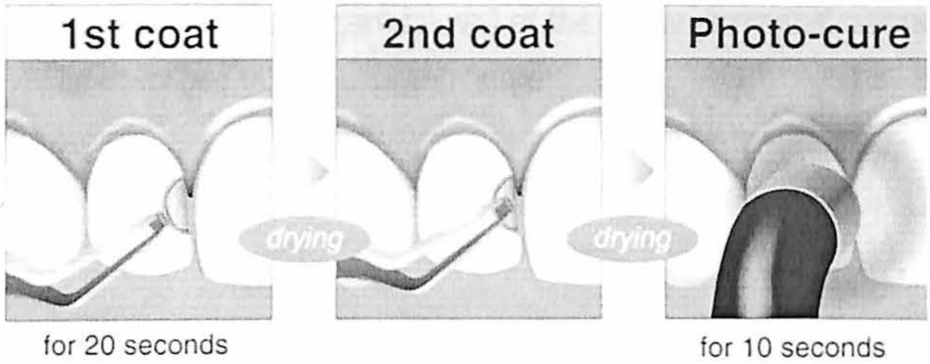

Fig. 2 Cavity treatment with Single-Step technique

Specimens were thermocycled between 5° and 55° C for 800 cycles to imitate the actual condition in the oral cavity by using water-bath machine. The dwelling time for each temperature are 20 seconds and another 20 seconds for transfer time. Evaluation of microleakage of composite restorations must include thermocycling in order to simulate intraoral condition (Kidd, 1985). Posterior composite restorations with occlusal surfaces can be loaded in addition to simulate masticatory forces (Raadal, 1979). Microleakage under these circumstances may yield a more realistic approximation of what may be expected *in vivo* (Kidd, 1985).

Specimens were then cut longitudinally using superfine double-sided diamond disc with mandrel placed on Amman AF350 cutting machine, to form a dimension of 2 mm thick and 10 mm in diameter.

Specimens were ground and polished on one side with fine grits of silicone carbide paper using Knuth Rotor water cooled polishing machine under 10,000 rpm. The prepared specimens were fixed to SEM ***stub*** using double-sided adhesive tape and observed on the Scanning Electron Microscope (SEM).

Photomicrographs were taken of representative areas (from 0 to 3 in the cavity walls) where area 1 is on enamel of the occlusal wall; area 2 is on dentin and area 3 is on enamel of cervical wall of the cavity at magnifications of 1.000 times as described in the following figure:

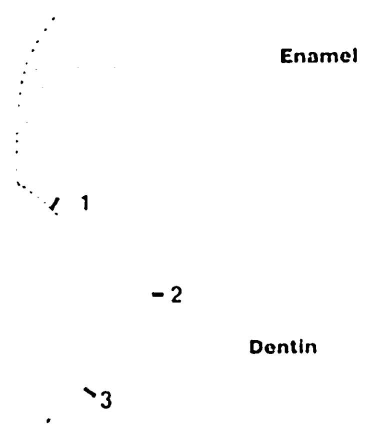

Fig. 3. Representative area on the enamel and dentin walls of the cavity

Scanning electron micrographs as well as measurement of micro leakage depth were made on 3 areas of the cavity as described in figure 3. The width of microleakage were measured (in μm) direct from the SEM devices as well as from the Photomigrograph and further qualitative and qualitative analysis was done.

Results

Measurement on Multi-Step technique showed the microleakage distance as on Table 2 that showed also the distance of microleakage in three areas of measurements.

Table 2. Microleakage distance on Multi-Step technique.

# SAMPLE	A.M. 1 (μm)	A.M. 2 (μm)	A.M. 3 (μm)	MEAN (μm)
1	0	5.75	0	1.9167
3	0	11.9	0	1.83
5	8.16	7.4	0	5.1867
7	0	9.81	9.63	6.48
9	19.1	11.7	0	10.2667
11	0	0	0	0
13	39.6	43.9	0	27.8333
15	0	0	10	3.3333
17	0	0	0	0
19	0	0	0	0
21	0	28.6	6.03	11.5433
23	0	8.42	0	2.8067
25	0	5.9	0	1.9667
27	0	8.6	6.4	5
29	0	5	0	1.6667
31	0	12.34	0	4.1133
33	0	0	0	0
35	11.8	13.6	0	8.4667
37	8.02	12.7	0	6.9067
39	12.54	18.68	0	10.4067
				109.7235

A.M. : Area of measurement

SEM 1000 x magnification

Measurement on Single-Step technique showed the microleakage distance as on Table 2 that showed the distance of microleakage in three areas of measurements.

Table 3. Microleakage distance on Single-Step technique.

# SAMPLE	A.M. 1 (μm)	A.M. 2 (μm)	A.M. 3 (μm)	MEAN (μm)
2	2.26	5.49	3.14	3.63
4	0	0	9.76	3.2533
6	4.22	4	2.44	3.5533
8	5.8	0	0	1.9333
10	0	0	0	0
12	0	15.2	19.6	11.6
14	0	0	0	0
16	0	0	0	0
18	0	0.62	0.58	0.4
20	0	8	0	2.6667
22	0	0	0	0
24	0	11.4	3.26	4.8867
26	0	11.9	0	3.9667
28	0	0	1.16	0.3867
30	0	6.81	4.78	3.8633
32	0	3.57	0	1.19
34	0	0	9.98	3.3267
36	0	0	0	0
38	0	0	0	0
40	0	0	0	0
				44.6567

A.M. : Area of measurement

SEM 1000 x magnification

Comparison quantitatively the degree of microleakage in Multi-Step with Single-Step techniques using Two-sample Wilcoxon rank-sum (Mann-Whitney) test showed Single-Step technique have less micro-leakage compared to Multi-Step technique, but statistically not significant as shown in Table 4.

Table 4. Comparison of microleakage in Multi-Step and Single-Step techniques.

Two-samples Wilcoxon rank-sum (Mann-Whitney) test

id	obs	rank sum	expected
1	60	3905	3630
2	60	3355	3630
combined	120	7260	7260

unadjusted variance	36300.00
adjustment for ties	-8511.68
adjusted variance	27788.32

Ho: width (id=1) = width (id=2)

z = 1.650

Prob > |z| = 0.0990

Z (1.650) < Z table (1.960) on $p<0.05$

[Not significant]

Comparison the degree of micro leakage in occlusal enamel; dentin and cervical enamel areas of the cavity in Multi-Step with Single-Step techniques is showed in Table 5, 6 and 7

Table 5. Comparison of microleakage distance on area 1 (Occlusal enamel area) between Single-Step and Multi-Step techniques:

Two-samples Wilcoxon rank-sum (Mann-Whitney) test

seal1	obs	rank sum	expected
0	20	449	410
1	20	371	410
combined	40	820	820

unadjusted variance	1366.67
adjustment for ties	-635.90
adjusted variance	730.77

Ho: width (seal1=0) = width (seal1=1)

z = 1.443

Prob > |z| = 0.1491

Z (1.443) < Z table (1.960) on $p<0.05$ [Not significant]

Same result also found using Two-samples t test with equal variances :

P>|t| = 0.0604> 0.05 [Not significant]

Table 6. Comparison of microleakage distance on area 2 (Dentin area) between Single-Step and Multi-Step techniques:

Two-samples Wilcoxon rank-sum (Mann-Whitney) test

seal1	obs	rank sum	expected
0	20	502	410
1	20	318	410
combined	40	820	820

unadjusted variance	1366.67
adjustment for ties	-87.31
adjusted variance	1279.36

Ho: width (seal1=0) = width (seal1=1)
Z = 2.572
Prob > |z| = 0.0101

Z (2.572) > Z table (1.960) on p<0.05 [Significant]

Same result also found using Two-samples t test with equal variances :

P>|t| = 0.0127< 0.05 [Significant]

Table 7. Comparison of microleakage distance on area 3 (Cervical enamel area) between Single-Step and Multi-Step techniques:

Two-samples Wilcoxon rank-sum (Mann-Whitney) test

seal1	obs	rank sum	expected
0	20	368	410
1	20	452	410
combined	40	820	820

unadjusted variance	1366.67
adjustment for ties	-420.00
adjusted variance	946.67

Ho: width (seal1=0) = width (seal1=1)
Z = -1.365
Prob > |z| = 0.1722

Z (1.365) < Z table (1.960) on p<0.05 [Not significant]

Same result also found using Two-samples t test with equal variances :

P>|t| = 0.4092> 0.05 [Not significant]

Discussion

In this study, Single-Step technique showed less total micro-leakage compared to Multi-Step technique, but statistically not significant. It is because of small sample size in study, according to the high cost of Scanning Electron Microscope (SEM) study. Bigger sample size could give different statistic result.

It was concluded in this study that Single-Step technique showed no different result in reducing microleakage compared to Multi-Step technique and therefore can be used as an alternative treatment in the clinic. This result similar with the study conducted by Garcia-Godoy (2000), who found the Single-Step adhesive (Prompt/ESPE) showed similar bond strength to the enamel and dentin with the Multi-Step adhesive. The bond strength of Single-Step adhesive was equivalent to the bond strengths obtained by Multi-Step adhesive (EXCiTE and Prime & Bond NT and there was no statistically significant difference between these adhesives when using hybrid composite (Z250) as the resin-based composite.

In the other clinical study, Bogoshian (2000) found that Single-Step adhesive systems (Prompt/ESPE) showed better adhesion to the tooth structure in the restoration of Class V lesion cavities compare to the Multi-Step adhesive (Elipar TriLight/ESPE). With the elimination of additional steps, including acid etching as a separate step, Single-Step adhesive allows clinician to perform adhesive restorative procedures in a time-efficient manner.

Comparison the degree of leakage in different area of measurement, showed interesting result. Microleakage in Occlusal and Cervical enamel showed no statistically significant differences between Single-Step and Multi-Step adhesives. Both adhesives showed good adhesion with an enamel and flows into interstices between and within enamel rods to form micro-tags and becoming interlocked with the surface irregularities created by etching. Indeed, resin-bonded enamel contains micro-tags of resin as small as 0.05 µm in diameter (Bayne and Taylor, 1995).

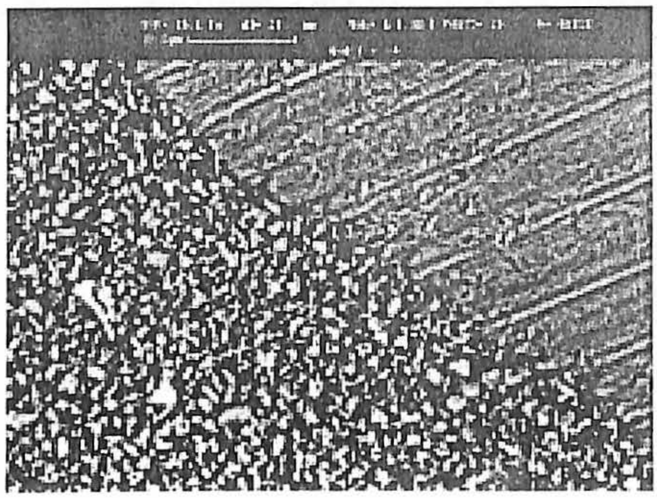

Fig. 4. Adhesives showed good adhesion with an occlusal enamel

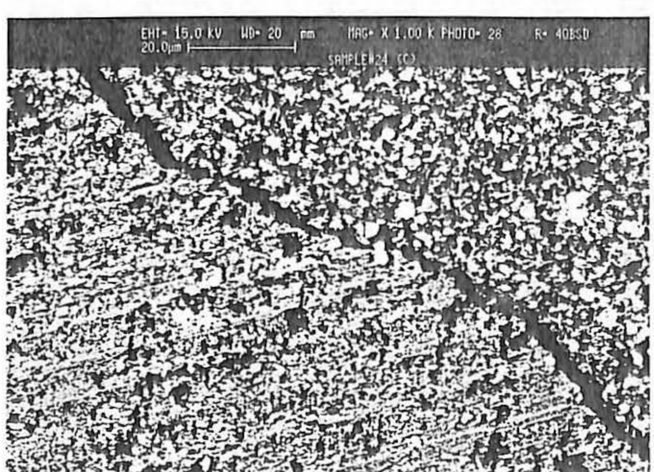

Fig. 5. Adhesives showed leakage with an enamel

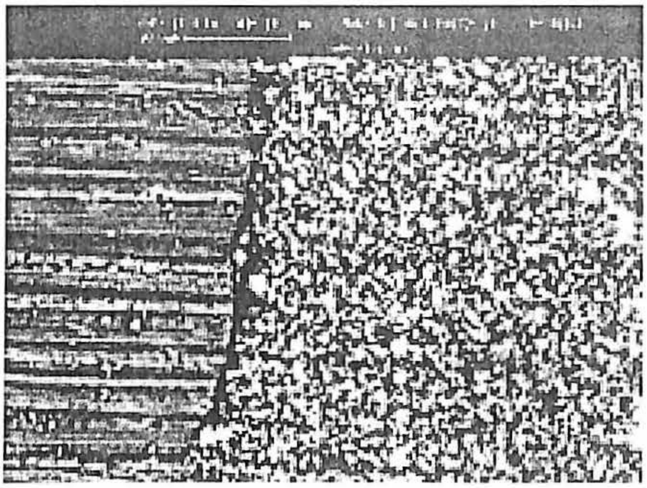

Fig. 6. Adhesives showed good adhesion with cervical enamel

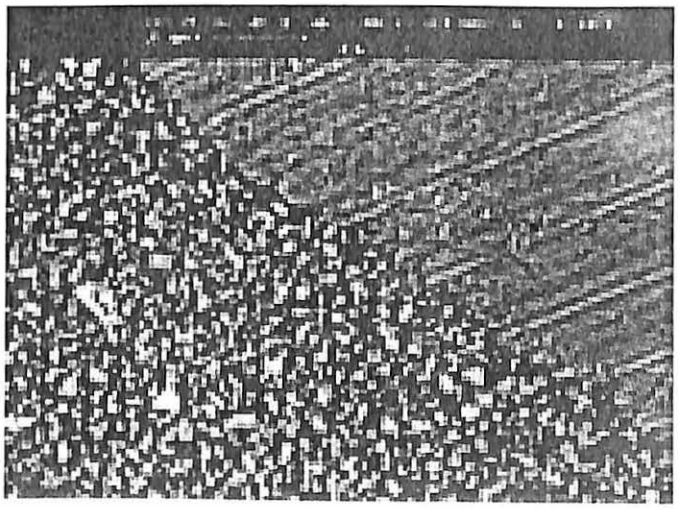

Fig. 7. Adhesives showed good adhesion with the dentin

The goals of enamel etching are to clean the enamel, to remove the enamel smear layer, to increase microscopic roughness (Retief *et al.*, 1986), by removal of prismatic and interprismatic mineral crystals. This action will increase the surface free energy of enamel (Busscher et al., 1987) to produce enough monomer infiltration to seal enamel surfaces with resin and to contribute to the retention of resin composite restorations.

When enamel is acid etched, it is transformed from a smooth, solid surface to microscopically rough, porous surface enamel by preferential solubilization of interprismatic rather than prismatic crystallites. The fact that some adhesion-promoting monomers permit deeper penetration of resin monomer to etched enamel indicates that the diffusivity of the resin co-monomers is very important. Nakabayashi and Pashley, 1998 found that 4-META showed 23 μm length of hybridization tags on enamel compared to 10 μm without adhesion monomers.

When resin monomers penetrate the microscopic porosities in enamel or dentin, they create 'enamel or dentin hybrid layer' and Nakabashi (1998) introduced this concept as 'hybrid concept'. The hybrid layer is resin-infiltrated enamel, dentin or cementum. The chemical and physical properties of these zones are very different from those of the original tooth structure, because it has been partially demineralized and then infiltrated with resin. The resulting structure is neither resin nor tooth but a hybrid of the two.

Hybrid layer is extremely important in the adhesion between resins to the mineralized tissues such enamel and dentin. When resin is placed close to tooth structure, no connection develops, because there is no biologically active soft tissue between the two structures to promotes regeneration. To solve this problem, a liquid must first be used to form an intimate contact between the material (monomer) and the solid hard tissue; then the liquid penetrates and is converted to a solid via polymerization. This was the approach that was selected in the attempt to unite biocompatible monomers with tooth structures. Single-Step as well as Multi-Step adhesive have confirmed following the approach very well as there were showed no differences in leakage depth between both adhesives on the enamel surfaces.

Acid etching of dentin is necessary to increase the porosity of intertubular dentin for monomer infiltration. The goal of a resin-dentin bonding resin is to attach composite resin to healthy dentin and to seal the dentin tubules against the entry of bacteria and their toxins. A successful union will also prevent both inward and outward flow of fluid from either the oral environment or the pulp. It is essential to retain this seal while curing the composite resin restoration because loss of bond will be likely to be associated wit post-restoration sensitivity, caries and loss of the restoration.

There are shown in this study that microleakage in Single-Step adhesive was significantly less compared to Multi-Step adhesive. It means the Single-Step adhesive have a better union with the dentin compare to Multi-Step adhesive and reduced microleakage in the dentin area.

In conventional, Multi-Step bonding procedures, an acidic etching is applied and rinsed off with water. The water is blown off (at the risk of drying or collapsing the demineralized dentinal mesh) and then primer is applied followed by bonding resin. If too little rinsing occurs, residual acid may over-etch the dentin or residual reaction products may block the narrow channels around the collagen fibrils. Self-etching or Single-Step systems may avoid this problems. The liquid is applied, allowed to react for 30 seconds and then air dried to remove the smeared layer.

Marshall *et al* (1995) reported that demineralization of intertubular dentin by etching procedures led to contraction or collapse of 0.3 to 0.5 μm of the matrix. This surface collapse is minor compared to very large, rapid shrinkage that occur when most demineralized dentin is air-dried (Nakabayashi, 1998).

There are at least two possible explanations for the major shrinkage of the demineralized dentinal meshwork when it is air-dried. The passive theory assumes that the demineralized collagen fibrils network is floating or suspended in water. Each fibril is separated from the other by a water-filled space, which occupies the space that was previously occupied by apatite crystallites. As the water-supported collagen network is air dried, the amount of water separating the fibrils disappears as the water evaporates, and the collagen fibrils come closer together in all three dimensions.

An alternative possibility is that the collagen fibrils develop a force as water is evaporated from the network. If collagen fibrils lose water from between constituent microfibrils, they may shorten slightly. However, because they are interconnected, this shortening of surface fibrils summates rapidly, causing the underlying soft, compliant network to be pulled down (Carvalho *et al.*, 1996).

As collagen molecules come closer together, they may interact by forming hydrogen bonds as well as interacting electrostatistically and hydrophobically. All of these events would cause more intermolecular interactions between collagen molecules than can normally occur in the presence of water. If water or an aqueous primer is added to dried dentin, the water reverses all of these events and plasticizes the fibrils, permitting the network to actively re-expand.

Kanca (1992) and Gwinett (1994) recommended that etched dentin not be dried before application of the bonding primer. Gwinnett at al. (1996) has demonstrated that moist bonding increases the bond strengths of many bonding systems. However, the water must be displaced from the collagen fibrils during bonding if each fibril is to be completely enveloped by resin. If too much water is present, the resin monomers may not be able to successfully compete for the collagen fibril surface, thereby leaving voids.

The ideal Single-Step adhesive is that can penetrate 2.0 µm of smear layer and engage underlying dentin to a depth of about 1.0 µm. This should provide sufficient retentive strength and adequate seal even if the infiltrated smear layer fails.

Conclusion

1. Single-Step technique showed no different result in reducing microleakage compared to Multi-Step technique and therefore can be used as an alternative treatment in the clinic.

2. Single-Step technique showed significant low microleakage in dentin compare to the Multi-Step technique.

3. Further study should be done to confirm biocompatibility and post-op tooth sensitivity between these two techniques.

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Appendix 1. Comparison analysis of microleakage distance on area 1 (Occlusal enamel area) between Single-Step and Multi-Step techniques:

1. Two-samples Wilcoxon rank-sum (Mann-Whitney) test

seal1	obs	rank sum	expected
0	20	449	410
1	20	371	410
combined	40	820	820

unadjusted variance 1366.67
adjustment for ties -635.90

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adjusted variance 730.77

Ho: width (seal1=0) = width (seal1=1)
Z = 1.443
Prob > |z| = 0.1491

Z (1.443) < Z table (1.960) on p<0.05 [Not significant]

2. Two-samples t test with equal variances

Group	Obs	Mean	Std. Err.	Std. Dev.	[95% Conf. Interval]	
0	20	4.961	2.217239	9.915796	.3202647	9.601735
1	20	.614	.3591513	1.606174	-.1377124	1.365712
combined	40	2.7875	1.161927	7.348674	.4372801	5.13772
diff		4.347	2.246139		-.2000705	8.89407

Degrees of freedom: 38

Ho: mean(0) - mean(1) = diff = 0

Ha: diff < 0	Ha: diff ~= 0	Ha: diff > 0
t = 1.9353	t = 1.9353	t = 1.9353
P < t = 0.9698	**P > |t| = 0.0604**	P > t = 0.0302

P>|t| = 0.0604> 0.05 [Not significant]

Appendix 2. Comparison analysis of microleakage distance on area 2 (Dentin area) between Single-Step and Multi-Step techniques:

1. Two-samples Wilcoxon rank-sum (Mann-Whitney) test

seal1	obs	rank sum	expected
0	20	502	410
1	20	318	410
combined	40	820	820

unadjusted variance 1366.67
adjustment for ties -87.31
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adjusted variance 1279.36

Ho: width (seal1=0) = width (seal1=1)
Z = 2.572
Prob > |z| = 0.0101

Z (2.572) > Z table (1.960) on p<0.05 [Significant]

2. Two-samples t test with equal variances

Group	Obs	Mean	Std. Err.	Std. Dev.	[95% Conf.	Interval]
0	20	10.215	2.391288	10.69417	5.209976	15.22002
1	20	3.3495	1.085639	4.855125	1.077232	5.621768
combined	40	6.78225	1.40789	8.90428	3.934523	9.629977
diff		6.8655	2.62619		1.549058	12.18194

Degrees of freedom: 38

Ho: mean(0) - mean(1) = diff = 0

Ha: diff < 0	Ha: diff ~= 0	Ha: diff > 0
t = 2.6142	t = 2.6142	t = 2.6142
P < t = 0.9936	**P > |t| = 0.0127**	P > t = 0.0064

P>|t| = 0.0127< 0.05 [Significant]

Appendix 3. Comparison analysis of microleakage distance on area 3 (Cervical enamel area) between Single-Step and Multi-Step techniques:

1. Two-samples Wilcoxon rank-sum (Mann-Whitney) test

seal1	obs	rank sum	expected
0	20	368	410
1	20	452	410
combined	40	820	820

unadjusted variance	1366.67
adjustment for ties	-420.00
adjusted variance	946.67

Ho: width (seal1=0) = width (seal1=1)

z = -1.365

Prob > |z| = 0.1722

Z (1.365) < Z table (1.960) on p<0.05 [Not significant]

2. Two-samples t test with equal variances

Group	Obs	Mean	Std. Err.	Std. Dev.	[95% Conf.	Interval]
0	20	1.603	.7585749	3.39245	.0152845	3.190715
1	20	2.735	1.124353	5.028261	.3817016	5.088298
combined	40	2.169	.6755172	4.272346	.8026376	3.535362
diff		-1.132	1.356321		-3.877728	1.613728

Degrees of freedom: 38

Ho: mean(0) - mean(1) = diff = 0

Ha: diff < 0	Ha: diff ~= 0	Ha: diff > 0
t = -0.8346	t = -0.8346	t = -0.8346
P < t = 0.2046	**P > |t| = 0.4092**	P > t = 0.7954

P>|t| = 0.4092> 0.05 [Not significant]

Appendix 4. Borang Maklumat dan Keizinan Pesakit

Borang Maklumat dan Keizinan Pesakit
Untuk Kajian : Uses of Conventional and Novel Expertise Development to Study Microleakage in Tooth Restoration using Composite Resin.

Untuk menyertai kajian ini, anda atau wakil sah anda mesti menandatangani mukasurat ini.

Dengan menandatangani mukasurat ini, anda mengesahkan yang berikut:

- Anda telah membaca semua maklumat dalam Borang Maklumat dan Keizinan Pesakit ini, dan anda telahpun diberi masa yang mencukupi untuk mempertimbangkan maklumat tersebut.
- Semua soalan-soalan anda telah dijawab dengan memuaskan.
- Anda, secara sukarela, bersetuju menyertai kajian penyelidikan ini, mematuhi segala prosedur kajian dan memberi maklumat yang diperlukan kepada doctor, para jururawat dan juga kakitangan lain yang berkaitan apabila diminta.
- Anda boleh menamatkan penyertaan anda dalam kajian ini pada bila-bila masa.
- Anda telahpun menerima satu salinan Borang Maklumat dan Keizinan Pesakit untuk simpinan peribadi anda.

____
Nama Pesakit (Ditera atau Ditaip)

____
Nama Singkatan dan
Nombor Pesakit

____
Tandatangan Pesakit atau Wakil Sah

____
Tarikh (ddMMyy)
(tambahkan masa jika sesuai)

____
Nama Individu yang Mengendalikan
Perbincangan Keizinan (Ditera dan Ditaip)

____
Tandatangan Individu yang Mengendalikan
Perbincangan Keizinan

____
Tarikh (ddMMyy)