FISSURE PENETRATION AND MICROLEAKAGE OF A CONVENTIONAL PIT AND FISSURE SEALANT AND A FLOWABLE COMPOSITE: A COMPARATIVE STUDY USING THREE DIFFERENT BONDING SYSTEMS

by

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Commendations are warranted for the tireless efforts of Dr. Jeffrey A. Plast and Dr. B. Keith Moore. Their understanding and patience has encouraged me to complete this thesis. Many thanks for Dr. Wm. Wye, who generously spent hours helping me to understand the data generated.

I would also like to give a special, heartfelt, blessing for my wife, Minghui, who has given me the strength and tenacity to follow my dreams. She is my inspiration for life.

I am indebted to Ms. Barbara Rhorer, who was the navigational force behind this research project. Ms. Rhorer has carefully guided me through all the switches, wires, and drills around the research laboratory.

I would also like to acknowledge my program director, Dr. Jeffrey A. Dean. His knowledge of Pediatric Dentistry and Endodontics has given me visions of excellence and has inspired me to pursue the same.

Special thanks are extended toward the rest of the committee for their help in supporting my efforts to further my education: Drs. David R. Avery, James A. Weddell, Angela Touzani and Brian J. Sanders.

Gratitude is given to the United States Navy for allowing me the opportunity to achieve this milestone. It is an honor to serve in a country where dreams are made into reality.
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The concept of using a pit and fissure sealant material to prevent dental caries may be historically linked to Wilson, who described the use of zinc phosphate cement to seal occlusal pits and fissures over 100 years ago. The sealing of these occlusal pits and fissures provided a physical barrier to the impaction of food and microorganisms, both of which contribute to the etiological factors in dental caries. This early attempt to reduce occlusal caries, however, did not provide long-term protection. It was 1955 before Buonocore demonstrated that using 15-per cent phosphoric acid to etch enamel for 30 seconds allowed for sufficient bonding between a resin sealant material and enamel. This acid-etch technique was designed to alter the tooth surface sufficiently for mechanical bonding of a low-viscosity acrylic resin material. This concept was based on an idea much like the industrial use of phosphoric acid preparations to treat metal surfaces for adhesion of paints and resin coatings.

Effectiveness of a pit and fissure sealant material is limited to its ability to remain bonded to the occlusal surfaces. The bonding capability of the acrylic resin depends on a clean and structurally microporous enamel surface produced by the acid etching. In addition, complete etching of the fissure wall enamel seems to be a crucial step in fissure sealing, since it could influence the long-term result by improving the seal and retention all the way to the bottom of the fissure. Failure to prevent contamination by moisture or saliva via the etched enamel surface has been cited as causing bond failure between the sealant material and the enamel surface. Adding a dentin-bonding agent between the etched enamel and the sealant material has been demonstrated as a way of optimizing...

INTRODUCTION
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Effectiveness of a pit and fissure sealant material is limited to its ability to remain bonded to the occlusal surfaces. The bonding capability of the acrylic resin depends on a clean and architecturally microporous enamel surface produced by the acid etching. In addition, complete etching of the fissure wall enamel seems to be a crucial step in fissure sealing, since it could influence the long-term result by improving the seal and retention all the way to the bottom of the fissure. Failure to prevent contamination by moisture or saliva on the etched enamel surfaces has been cited as causing bond failure between the sealant material and the enamel surfaces. Adding a dentin-bonding agent between the etched enamel and the sealant material has been demonstrated as a way of optimizing
bond strength in the face of moisture and salivary contamination, thus increasing sealant success. Another factor considered in the effectiveness of a sealant material is the ability to adequately penetrate and seal the pits and fissures to prevent microleakage, because microleakage may support the caries process beneath the sealant. Other studies have also confirmed the benefit of a dentin-bonding agent, such as using bonding agent under sealants to enhance the flow of resin into the fissures. Although not clinically detectable, the limitation to resin penetration is limited to the prismatic surface on enamel. It is reasonable to deduce that prismatic enamel allows a greater penetration of resin than does the prismless type. Prismatic enamel displayed larger pores in either rod cores or at rod peripheries when compared with prismless enamel, thus allowing resin penetration into the conditioned enamel. The sealant's ability to penetrate is also determined by the viscosity of the sealant material. Increases in the viscosity of the sealant by addition of filler particles will lower its penetration coefficient. It has been demonstrated that the penetrativity of pit and fissure sealants can be quantified in terms of the penetration coefficient, which has been shown as a function of the properties of the sealant: surface tension, viscosity, and contact angle on the capillary wall. In addition, sealants tend to fail to completely penetrate into fissures that are deep and narrow, while sealants will adequately penetrate in fissures that are shallow and wide.

Microleakage around a pit and fissure sealant has been shown to support the carious process within fissures. It has also been demonstrated even when there was good penetration of the sealant that microleakage still exists. Although absence of marginal leakage or microleakage of the enamel-sealant interface determines the caries-reduction ability of a pit and fissure sealant, it has been demonstrated that sealants can
prevent the progression of carious lesions toward the dentinoenamel junction through the
presence of sealant tags projecting into the pores created by acid-etching of the enamel. It has also been suggested that even though leakage may be taking place beneath the sealant, food particles and plaque cannot gain access to the pit and fissure areas with the sealant in place. To demonstrate microleakage in vitro, it has been reported that through thermal cycling tests, hot water may accelerate hydrolysis of the resin as well as the bonding agent to enable evaluation of potential leakage. However, the number of thermocyclings, the immersion period and the bath temperature have not been standardized to date.

The rationale for the addition of filler particles by manufacturers in their sealants was to increase wear and abrasion resistance. Filler content is a description of the quantity of filler in a resin. It is measured as the weight:weight quantity of the filler placed into the resin matrix and is expressed as a percent. Filled sealant can be described as being filled approximately 30-percent by weight. Concerns about occlusal wear and abrasion have resulted in the use of flowable composites as potential pit and fissure sealant materials. Flowable composites are composite-resin materials that are 50-percent to 70-percent filled by weight. This increase in filler particles does not seem to lower a sealant material’s retention. This low-viscosity resin composite has the desirable handling property that allows the material to be used in an amplified range of application, such as in pit and fissure sealant.

The purpose of this in vitro study was to compare the fissure penetration and microleakage of a conventional pit and fissure sealant and a flowable composite used as a sealant, while using three different bonding systems: a total etch system - utilizing only
phosphoric acid etch; a single-bottle bonding system - one bottle containing both the primer and adhesive but requiring a separate phosphoric acid etch; and an all-in-one self-etching primer/adhesive system.
Prevention and control of dental caries in primary and permanent teeth is a primary objective of dental practitioners. One preventive technique is the use of pit and fissure sealants. Successful application of pit and fissure sealants was first reported by Cuello and Buonocore in 1967. This preventive procedure and its efficacy in managing dental caries have been further established by scientific research. Basically, the sealing of these occlusal pit and fissures provided a physical barrier to the impaction of food and microorganisms, both factors contribute to the etiological factors in dental caries. Since plaque in fissures takes about 2 to 4 weeks to mature and completely fill the fissures, a deeper bacterial penetration is more likely to occur in the period immediately after tooth eruption. Currently, the guideline is that sealants are intended to protect caries-susceptible tooth surfaces and should be placed as soon as possible after the tooth erupts, and isolation to prevent moisture contamination can be obtained. Additionally, it has been reported that permanent mandibular molars were significantly more frequently decayed or restored than the molars in the maxilla. Moisture control particularly concerning the buccal pits of the mandibular molars was cited as a leading cause of failed sealants. Usage of pit and fissure sealants, however, is not as high as hoped. According to a report from the National Institutes of Health, pit and fissure caries accounted for 98 percent of the total caries experienced by school children between 1986 and 1987. Current reports of usage indicates that only 18.5 percent of US children between ages 5 and 17 years had never more sealed permanent teeth. Low utilization could be
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Usage of pit and fissure sealants, however, is not as high as hoped. According to a report from the National Institutes of Health, pit and fissure caries accounted for 88-percent of the total caries experienced by school children between 1986 and 1987. Current reports of usage indicate that only 18.5 percent of US children between ages 5 and 17 years had one or more sealed permanent teeth. Low utilization could be
attributed to the various factors that influence practitioners’ decisions. Performing the service of a pit and fissure sealant requires the practitioner to make decisions on how to prepare the fissure (if preparation is at all indicated), which bonding agents to use (if a bonding agent is indicated), which sealant to use, how to place the sealant, and how to maintain the sealant. More specifically, significant reported reasons for low utilization of sealants have been attributed to a lack of confidence in the bonding of sealant to enamel and to the difficulty of achieving adequate moisture control. Furthermore, long-term studies have documented retention rates for resin sealants for only as long as four years after placement.

In order to ease practitioners’ lack of confidence in the bonding of sealant to enamel, they must first acquire an understanding of the acid-etch technique. It is this technique that enables the pit and fissure sealants to be bonded to the enamel. The acid-etch technique is actually conditioning of the enamel, which produces a roughened surface by lifting off organic pellicles and dissolving thin calcific deposits. Buonocore reported numerous rationales for the success of the acid-etch technique such as: the phosphoric acid preparations used to treat enamel surfaces 1) produced increased surface areas and exposed the organic framework of enamel, which then served as a network to which the acrylic resin could adhere; 2) removed old fully reacted and inert enamel surface and exposed fresh reactive surface more favorable for bonding, and 3) created a strongly absorbed layer of highly polar phosphate groups derived from the acid. This process involves a series of clinically established steps to include acid etching, water rinsing, and air drying, prior to the application of the pit and fissure sealant materials. Originally, Buonocore found that acrylic resin could bond to the enamel when 85-
percent phosphoric acid was used for 30 seconds. Today, most commercially available etchants contain 30-percent to 40-percent phosphoric acid, which provides enamel surfaces with the most retentive appearance. This has become a standard procedure for surface conditioning of enamel prior to the bonding agent application.

Due to its fluid property, when an acrylic resin is applied over the treated enamel surfaces, it appears to penetrate into the spaces created by the phosphoric acid. It was later suggested that these spaces or resin tags were providing the primary attachment mechanism of the acrylic resin to the acid-etched enamel. This mechanical retention has been described as strong capillary action of the microspaces leading to penetration of the resin into the microscopic capillaries, voids and crevices which serve to hold the resin firmly in close proximity to the enamel surface. This acid-etch technique has been the foundation behind the ability of the acrylic resin material to be bonded in the pit and fissures. The acrylic resin material later evolved into the pit and fissure sealant materials used today.

The acrylic resins that bond to the enamel are generally based on bis-phenol A glycidyl dimethacrylate (BIS-GMS) with the addition of diluents (such as triethyleneglycol dimethacrylate). Polymerization of the dimethacrylate monomer material is accelerated by visible light (420 to 450 nm wave length). Camphoroquinone is used as the photoinitiator sensitive to the visible light.

In spite of the presence of two hydroxyl groups, the BIS-GMA monomer is insufficiently hydrophilic to compete with water for interaction with the enamel surface. Water within the microscopic capillaries would prevent complete filling of the acrylic resin. Contamination of the etched enamel surfaces with saliva prior to sealant
application will prevent proper bonding, because the micropores become occluded. Thus, moisture contamination of etched enamel during application of sealant is the most frequently cited reason for sealant failure. Preliminary studies on the use of a dentin bonding agent under sealants as an intermediate layer has been shown to significantly increase bond strength on wet, contaminated enamel.

Because dentinal bonding agents were developed to bond restorations to a continuously wet tissue, dentin, it was hypothesized that these agents may allow bonding to wet enamel surfaces as well. These dentinal bonding agents are bifunctional molecules with a methacrylate group that bonds to the restorative resin by chemical interaction and a functional group that bonds to either the inorganic or organic constituents of dentin.

Traditionally, the dentinal bonding system consists of three steps: (1) etching, (2) priming, and (3) bonding. Initially, the etching, commonly an inorganic acidic component, removes the smear layer, opens the dentinal tubules, increases dentin permeability, decalcifies the intertubular and peritubular dentin, and increases the microporosity of the intertubular dentin. After the etchant is rinsed off, a primer consisting of a solvent with one or more hydrophilic resin monomers is then applied. Primer molecules contain two functional groups - a hydrophilic group and a hydrophobic group. The hydrophilic group has an affinity for the dentin surface and the hydrophobic (methacrylate) group has an affinity for resin. The primer wets and penetrates the collagen meshwork, raising it almost to its original level. The primer also increases the surface energy, and hence the wettability, of the dentin surface. Finally, an unfilled resin, a dimethacrylate resin monomer, is applied and penetrates into the primed dentin,
copolymerizing with the primer to form an intermingled layer of collagen and resin commonly called the hybrid layer.\textsuperscript{43} Formation of this hybrid layer of dentin and resin is thought to be the primary bonding mechanism of most current adhesive systems.\textsuperscript{44}

However, the three steps have been perceived as time-consuming and may contribute to confusion and be prone to errors of application. Sensing this, many manufacturers have attempted to simplify the bonding system by combining certain steps. Initially, the single-bottle system has been an attempt to combine the primer and bonding agent into a single-bottle. This process would still require the separate step of acid etching of the enamel or dentin. Although these single-bottle systems are promoted as simplified bonding, investigators have reported bond strengths values similar to those of the conventional three-step systems while other have reported lower values.\textsuperscript{45,46}

Variations amongst these single-bottle systems may be related to their composition. Recent work suggests that water-based primers or adhesives may be less effective in bonding to etched surfaces than are acetone-or ethanol-based adhesive systems.\textsuperscript{47,48}

Current trends in development of adhesive have lead to further simplifying of the bonding system. Some manufacturers have introduced the idea of combining the three steps of the dentinal bonding system into a truly single step, thus eliminating the pretreatment or conditioning of the enamel with a separate phosphoric acid. Known as self-etching primers, manufacturers introduced the use of hydrophilic, acidic monomers capable of etching and penetrating the enamel simultaneously. These self-etching primers can form hybrid layers that approach the thickness of those derived from a separate phosphoric acid etching step.\textsuperscript{49} Monomers with these properties are employed in various dentin adhesives as a single product. The reactive components in self-etching
primers are esters from bivalent alcohols with methacrylic acid and phosphoric acid or derivates. The phosphate residue is thought to etch the enamel, while the methacrylate component of the molecule is available for copolymerization with the bonding agent and resin. With this process, there is no need to rinse off reaction products or residual phosphoric acid ester, because both are subsequently polymerized into the bonding layer. The combination of the demineralizing agent with the hydrophilic primers should allow for a completely diffused hybrid layer that provides a strong and stable bond. Self-etching primers have been suggested to be an effective alternative to conventional phosphoric acid etchants in conditioning the enamel surface to secure a durable conditioning and marginal seal of resin restorations. However, in a recent study the benefit of using self-etching primers in terms of simplifying the clinical procedure might be negated by the reduction in bond strength, which was demonstrated by thermal cycle testing. Another study demonstrated that self-etching primers contain a water content of over 70 percent and incomplete removal of water from the collagen network resulted in the competition between the monomer and inhibited polymerization of the bonding agent.

These hydrophilic dentin bonding agents originally designed to be used on moist enamel and dentin to increase retention of resin have been reported to improve retention for pit and fissure sealants as well. In a study by Tulunoglu et al. they reveal that the use of an enamel-dentin bonding agent as an intermediate layer between the primary tooth and fissure sealant would increase the bond strength and decreased the potential for microleakage. Without the use of a dentin bonding agent, contamination such as water or saliva greatly reduces the bond strength of the surface to resin. Investigations into the
effect of moisture on etched enamel surfaces have shown that the bond strength reduction is due to the altered microstructure of the etched surface as seen by scanning electron microscopy.\textsuperscript{58} Adhesion of the sealant material is thus significant to the success in prevention of caries. When lack of adhesion exists, then microleakage occurs.

It has also been suggested that mechanical forces will increase wear and abrasion to an unfilled resin.\textsuperscript{59} In addition, it has been demonstrated that breakage of the material through excessive wear will display leakage around a restoration.\textsuperscript{60} Filler particles were thus added by manufacturers to increase wear and abrasion resistance. Wear resistance increases when small, highly packed filler particles protect the polymer matrix in the resin from food bolus wear.\textsuperscript{61} Flowable composites were created by retaining the same small particle sizes of traditional hybrid composites, but reducing the filler content and allowing the increased resin to reduce the viscosity of the mixture.\textsuperscript{62} It has been suggested that flowable composites demonstrated favorable wear resistance, which would indicate it as an acceptable filling material in low-stress applications such as pit and fissure sealing.\textsuperscript{63} In a recent study, flowable composites demonstrated complete leakage resistance at enamel margins.\textsuperscript{64} Because flowable composites contain 50-percent to 70-percent filler particles, they should be considered as potential pit and fissure sealant materials.

Increased usage of in-office bleaching and home bleaching has been cited to reduce bond strengths of dental adhesive. It has been suggested that concentrated solutions of hydrogen peroxide, 30- to 35-percent, will reduce the bond strengths of dental adhesives.\textsuperscript{65} A method of cleansing the occlusal surfaces prior to sealant placement by using hydrogen peroxide has been advocated. This was considered a
potential interaction with bond strengths. However, the preparation used clinically was a negligible 2.0-percent hydrogen peroxide. Thus, no interactions has been found at the 2.0-percent level. 66

The purpose of this in vitro study was to compare the fissure penetration and microleakage of a conventional pit and fissure sealant and a flowable composite used as a sealant, while using three different bonding systems: a total etch system -- utilizing only phosphoric acid etch; a single-bottle system -- one bottle containing both the primer and adhesive but requiring a separate phosphoric acid etch; and an all-in-one self-etching primer/adhesive system.

Binary responses arise in many fields of study. Logistic regression analysis is often used to investigate the relation between these discrete responses and a set of explanatory variables. In this study, the effects of the pit and sealant materials and the adhesive systems on fissure penetration and microleakage were evaluated using logistic regression models. Before making the comparison among the different materials and adhesive systems, a logistic regression model was fit to test for the significant effects of material, adhesive system and their potential interaction. Effect was considered to be statistically significant if the p-value was less than 0.05.

The hypothesis of this study was that there would be no significant difference in fissure penetration or microleakage between the conventional pit and fissure sealant or the flowable composite when used as a pit and fissure sealant, regardless of the adhesive system used.
MATERIALS AND METHODS

The materials selected as pit and fissure sealants for this study were Delton Direct Delivery System (Dentsply, York, PA, Lot #105307) and Revolution Formula 2 (Kerr, Orange, CA, Lot # 104700). Delton DDS (Dentsply) is an unfilled, low viscosity, opaque, light-cured pit and fissure sealant and was used as the conventional sealant. Revolution Formula 2 (Kerr) is a commercially available, flowable, light-cured hybrid composite which contains 60 percent glass filler and was selected as the flowable composite for this study. To assist in visual detection, shade A3.5 was used.

The three clinically used adhesive systems selected for this study were: (1) the total etch system Delton Bond (Dentsply) (218), an all-purpose 34-percent phosphoric acid etchant; (2) the single bottle system consisting of a 37.5-percent phosphoric acid etchant application followed by the application of a mixture of adhesive/primer, which was OptiBond Solo Plus (Kerr, Orange, CA, Lot # 104022) a 75-percent filled, fluoride-releasing bonding agent designed for bonding all direct and indirect restorative materials; and (3) an all-in-one adhesive system that eliminates the need for separate etching, priming and bonding: Prompt L-Pop (3M ESPE, Plymouth, Minnesota, PA, Lot # 106139), used for direct, light-cured composites and composites, was used as the all-in-one adhesive system.

SPECIMEN PREPARATION

One hundred fifty extracted, caries-free, human third molars, selected for well-defined occlusal pits, were randomly divided into six treatment groups. Occlusal pits
The materials selected as pit and fissure sealants for this study were Delton Direct Delivery System (Dentsply, York, PA, Lot # 10507) and Revolution Formula 2 (Kerr, Orange CA, Lot # 108700). Delton DDS (Dentsply) is an unfilled, low viscosity, opaque, light cured pit and fissure sealant and was used as the conventional sealant. Revolution Formula 2 (Kerr) is a commercially available, flowable, light-cured hybrid composite which contains 60-percent glass filler and was selected as the flowable composite for this study. To assist in visual detection, shade A3.5 was used.

The three clinically used adhesive systems selected for this study were: (1) the total etch system: Delton EZ Etch (Dentsply, York, PA, Lot # 1218), an all-purpose 34-percent phosphoric acid etch; (2) the single bottle system: consisting of a 37.5-percent phosphoric acid etch application followed by the application of a unidose of adhesive/primer, which was Opti-bond Solo Plus (Kerr, Orange, CA, Lot # 104022) a 15-percent filled, fluoride-releasing bonding agent designed for bonding all direct and indirect restorative materials; and (3) the all-in-one adhesive system that eliminates the need for separate etching, priming and bonding: Prompt L-Pop (3M ESPE, Plymouth Meeting, PA, Lot # 106339), used for direct, light-cured composites and compomers, was used as the all-in-one adhesive system.

SPECIMEN PREPARATION

One hundred fifty extracted, caries-free, human third molars, selected for well-defined occlusal pits, were randomly divided into six treatment groups. Occlusal pits
were considered well-defined if recognizable landmarks were present. For example, if small pinpoint depressions were located at the junction of developmental grooves or at terminals of those grooves then they would be selected. Other landmarks referenced during the selection were the fossa, sulcus and developmental groove. Although there are variations among mandibular and maxillary third molars no attempt was made to separate the two, they were randomly distributed into the six treatment groups. Prior to placement of adhesive and sealant materials, fissures from each group of teeth were cleaned with a tapered prophylaxis brush dipped in 2.0-percent hydrogen peroxide and run at approximately 500 rpm for 10 seconds. Cleaned surfaces were flushed with an air-water spray for five seconds and lightly dried to eliminate visible moisture. To ensure proper delivery and material placement, the manufacturer's recommendations for all materials used were strictly followed. In addition, to maintain control and consistency only five teeth, mounted in compound material, were treated at a time.

ADHESIVE SYSTEMS

The "total etch" system Delton EZ Etch (Densply) is a 34-percent phosphoric acid tooth conditioner gel. The etchant was applied to the cleaned enamel and allowed to remain in place for 15 seconds. Following which, the conditioned areas were rinsed thoroughly with water for approximately 10 to 15 seconds and air dried with clean, oil-water-free compressed air for 15 seconds. This produced a dull, frosty white appearance to the properly conditioned enamel. Application of the pit and fissure sealant material was then performed.

For the single-bottle system OptiBond Solo Plus (Kerr) application was as directed. A 37.5-percent phosphoric acid (Kerr) was applied to the enamel for 15
seconds. Then, the conditioned areas were rinsed thoroughly with water for approximately 10 to 15 seconds and air dried with clean, oil-water-free compressed air for 15 seconds. This produced a dull, frosty white appearance to the properly conditioned enamel. OptiBond Solo Plus (Kerr) was then applied to the enamel surfaces with the applicator tip for 15 seconds, using a light brushing motion and air thinned for 3 seconds. The adhesive was light cured for 20 seconds. Application of the pit and fissure sealant material was then performed.

In the all-in-one adhesive system, squeezing the material from the red reservoir toward the disposable applicator activates Prompt L-Pop (3M ESPE). The red reservoir, which contains methacrylated phosphoric acid derivatives (esters), photosensitizers and stabilizers, was then squeezed until all the fluid was transferred to the yellow section. The yellow part contains water and soluble fluoride components. The red and yellow sections were then combined. Both these sections were then squeezed until the liquid was in the green section. Squeezing the green section transferred the liquid into the open elongated channel that houses the applicator tip. The solution was rubbed evenly into the entire enamel surface; it was during this step the tooth surface was simultaneously etched, conditioned, and coated with the bonding agent. A stream of air was then used to evenly disperse the material into a thin film. The surface appeared smooth and glossy, which indicated the presence of bonding agent. The Prompt L-Pop (3M ESPE) material was then light cured for 10 seconds. Application of the pit and fissure sealant material was then performed.
PIT AND FISSURE SEALANT MATERIAL

Application of Delton Pit and Fissure Sealants Direct Delivery System (Dentsply) was accomplished by dispensing the material onto a dappen dish and brushing the material onto the surfaces to be sealed with a micro brush and then light curing for 20 seconds with a light polymerization unit.

Application of Revolution Formula 2 (Kerr) was accomplished by using the dispensing syringe to brush the material onto the surfaces to be sealed and then light curing for 40 seconds with a light polymerization unit.

LIGHT POLYMERIZATION UNIT

The visible light activation unit used for this study was Spectrum™ 800 (Dentsply, York, PA). A built-in digital radiometer was used for precise output monitoring. Calibration for output was maintained at 450 mw/cm².

TREATMENT GROUPS

The six treatment groups were the following (Table I): Group I fissures received the Delton EZ Etch (Dentsply) total etch adhesive system and Delton DDS (Dentsply) sealant. Group II fissures received the Opti-bond Solo Plus (Kerr) single-bottle adhesive system and Delton DDS (Dentsply) sealant. Group III fissures received the Prompt L-Pop (3M ESPE) all-in-one self-etching primer/adhesive system and Delton DDS (Dentsply) sealant. Group IV fissures received the Delton EZ Etch (Dentsply) total etch adhesive system and Revolution Formula 2 (Kerr) flowable composite. Group V fissures received the Opti-bond Solo Plus (Kerr) single-bottle adhesive system and Revolution Formula 2 (Kerr) flowable composite. Group VI fissures received the Prompt L-Pop (3M
ESPE) all-in-one self-etching primer/adhesive system and Revolution Formula 2 (Kerr) flowable composite.

FISSURE PENETRATION AND MICROLEAKAGE ASSESSMENT

After pit and fissure sealant placement of Delton DDS (Dentsply) or Revolution Formula 2 (Kerr) all 150 teeth received 2,500 thermocycles between 5 °C and 45 °C water baths with a 30-second dwell time and a 10-second transit time. The teeth then received two alternating layers of fingernail varnish, which was placed beneath and above the .001 inch tin foil prior to immersion in a solution containing 5.0-percent methylene blue. Sealing with two alternating layers of fingernail varnish and tinfoil eliminated penetration of the dye into the cementum. After 18 hours, the teeth were removed from the dye and rinsed under running tap water for one hour and thoroughly brushed with detergent solution before and after the tin foil was removed.

Fissure penetration and microleakage were examined and measured twice by a single evaluator once from the mesial and once from the distal flat-ground sections made with wet 400-grit silicon carbide papers to locate fissures representative of adequate fissure depth in all 150 teeth. The ground sections were then examined at X20. Microleakage was recorded as either present or absent (1 for presence and 0 for absence). Penetration was recorded as either complete or incomplete (1 for complete and 0 for incomplete).

STATISTICAL ANALYSIS

The effects of the pit and fissure sealant material and the adhesive system on fissure penetration and microleakage were evaluated using logistic regression models.
Before making the comparison among the different materials and adhesive systems, a logistic regression model was fitted to test for the significant effects of the pit and fissure sealant material, adhesive system and their potential interaction. In order to improve the accuracy of the measurement, each response was measured twice by a single evaluator: once from the mesial and once from the distal flat-ground sections. If either one of the responses from the same tooth showed incomplete penetration, the response was treated as incomplete penetration. If either one of the responses from the same tooth presented microleakage, the response was treated as presenting microleakage.
Tables II and III list the data collected for penetration and microleakage for the specimen in each group.

Table IV presents the results of the logistic regression model for penetration and the tests of the main variables, materials, and adhesive systems, on penetration. As shown in Table IV, neither has a significant effect with p-values of 0.0477 and 0.056 for the materials and the adhesive systems, respectively.

Table V presents the results of the logistic regression model for microleakage and the tests of the main variables, materials, and adhesive systems, on microleakage. As shown in Table V, only the adhesive system has a significant effect with p-values of 0.0547 and 0.0029 for materials and adhesive systems, respectively. A pairwise comparison between the total-etch and the single-bottle adhesive showed no significant difference with a p-value of 0.277.

The logistic regression model can be used to calculate the probability of incomplete penetration or microleakage for each of the treatment groups. These are shown in Table VI for the penetration and Table VII for the microleakage. Tukey's pairwise comparisons were used for testing for the differences between the probabilities shown in Tables VI and VII. Groups which exhibited significant differences are marked with the * in these tables.
Tables II and III list the data collected for penetration and microleakage for the specimen in each group.

Table IV presents the results of the logistic regression model for penetration and the tests of the main variables, materials and adhesive systems, on penetration. As shown in Table IV, neither has a significant effect with p-values of 0.6477 and 0.096 for the materials and the adhesive systems, respectively.

Table V presents the results of the logistic regression model for microleakage and the tests of the main variables, materials and adhesive systems, on microleakage. As shown in Table V, only the adhesive system has a significant effect with p-values of 0.0547 and 0.0029 for materials and adhesive systems, respectively. A pairwise comparison between the total-etch and the single-bottle adhesive showed no significant difference with a p-value of 0.577.

The logistic regression model can be used to calculate the probability of incomplete penetration or microleakage for each of the treatment groups. These are shown in Table VI for the penetration and Table VII for the microleakage. Tukey’s pairwise comparisons were used for testing for the differences between the probabilities shown in Tables VI and VII. Groups which exhibited significant differences are marked with the * in these tables.
### TABLE I

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<td>Single-bottle</td>
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<tr>
<td>III</td>
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<td>VI</td>
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**TABLES AND FIGURES**
TABLE I

Six treatment groups

<table>
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<th>Adhesive System</th>
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TABLE II

Data collected from groups I – III

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Legend

Penetration
0 = no penetration
1 = penetration

Microleakage
0 = no leakage
1 = leakage
TABLE III

Data collected from groups IV – VI

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Legend
0 = no penetration
1 = penetration
0 = no leakage
1 = leakage
### TABLE IV
Logistic regression model for penetration

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<td>All-in-one *</td>
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*Reference category

Summary table for the main effects of materials and adhesive systems on penetration

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<th>Source</th>
<th>Degrees of freedom</th>
<th>Chi-Square</th>
<th>Pr&gt;ChiSq</th>
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### TABLE V

Logistic regression model for microleakage

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*Reference category

Summary table for the main effects of materials and adhesive systems on microleakage

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TABLE VI

Probability of having complete penetration from different materials and adhesive systems

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* Significantly differs from the others
TABLE VII

Probability of presenting microleakage from different materials by different adhesive systems

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<td>All-in-one</td>
<td>0.49</td>
<td>0.78</td>
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* Significantly differs from the others
FIGURE 1. Complete penetration and absence of microleakage.
FIGURE 2. Incomplete penetration and presence of microleakage.
DISCUSSION

Adequate penetration of a pit and fissure sealant material ensures a physical barrier to the invasion of food and microorganisms. It has been suggested that viscosity of the sealant material may determine its ability to penetrate. The results of the study indicate that there was no significant difference in penetration between a conventional pit and fissure sealant and flowable composite used as a pit and fissure sealant material. This contradicts the results of Perzina et al. that sealant materials with a low viscosity had a greater potential to penetrate into the fissures and the microporosities produced in the enamel by etching.[4] In addition, there was no significant difference in penetration among the different adhesive systems used with the sealant materials. A factor not considered in this study was the wetting properties of the sealant used within the adhesive system, which may contribute to the penetration of the sealant materials. It has been indicated that surface-active containing sealants with a low viscosity can penetrate completely into fissures and can produce an increased resistant and washable surface, which significantly increases sealant penetration into deep fissures.[4] Fissure morphology is another factor in determining the ability of a sealant material and adhesive to penetrate. Deep-narrow types of fissures may retain plaque and debris, which hamper the ability of a sealant material and adhesive to penetrate.[20]
PENETRATION

Adequate penetration of a pit and fissure sealant material ensures a physical barrier to the impaction of food and microorganisms. It has been suggested that viscosity of the sealant material may determine its ability to penetrate. The results of the study indicate that there was no significant difference in penetration between a conventional pit and fissure sealant and flowable composite used as a pit and fissure sealant material. This contradicts the results of Percinot et al. that sealant materials with a low viscosity had a greater potential to penetrate into the fissures and the microporosities produced in the enamel by etching.\textsuperscript{14} In addition, there were no significant differences in penetration among the different adhesive systems used with the sealant materials. A factor not considered in this study was the wetting properties of the etchant used within the adhesive system, which may contribute to the penetration of the sealant materials. It has been indicated that surfactant-containing etchants with a low viscosity can penetrate completely into fissures and can produce an increased retentive and wettable surface, which significantly increases sealant penetration into deep fissures.\textsuperscript{4} Fissure morphology is another factor in determining the ability of a sealant material and adhesive to penetrate. Deep-narrow types of fissures may retain plaque and debris, which hamper the ability of a sealant material and adhesive to penetrate.\textsuperscript{25}
MICROLEAKAGE

When evaluating microleakage in this study, dye penetration appeared to be present in all groups. This indicated that all groups present microleakage. A previous study suggested that dye penetration tended not to be through the material but along the enamel/sealant interface. In previous studies, microleakage seemed to be associated with a thick layer of bonding agent; the thickness of the adhesive layer, therefore, affects the quality of the adhesion. Although microleakage occurred in both materials studied, pit and fissures sealant may still prevent impaction of debris into the fissures. Therefore, we should not completely discount the value of a pit and fissure sealant. No significant differences in microleakage between a conventional pit and fissure sealant and flowable composite were found when using the total-etch and single-bottle adhesive system.

MICROLEAKAGE OF THE THREE ADHESIVE SYSTEMS

The microleakage associated among the three adhesive systems was also evaluated. There was a significant difference in microleakage among the three adhesive systems when adjusted for the materials used.

The all-in-one adhesive system displays increased microleakage in this in vitro study. Explanation for microleakage can be found from findings made in the study by Silva Telles et al. They concluded that the all-in-one adhesive system, Prompt-L-Pop (3M ESPE) failed to generate sealed interfaces consistently between the primary and permanent teeth and the composite resin or the compomer they evaluated. Another study demonstrated that when using Prompt-L-Pop it was necessary to repeat the application of the bonding systems up to five times to achieve the shiny surface that should have been
observed after one initial application according to the manufacturers’ instruction. However, in a recent in vitro study by Gillet et al., they demonstrated that the all-in-one adhesive system Prompt-L-Pop was as efficient as phosphoric acid in obtruding the fissures on non-carious bicuspid teeth with a flowable composite. Prompt-L-Pop has also been demonstrated to be aggressive enough to produce mild differential dissolution of the enamel crystallites for micro-mechanical retention and is comparable to the etching effect of phosphoric acid on intact, unground enamel. Further studies both in vitro and in vivo are recommended.

FACTORS RELATED TO THE SUCCESS OF PIT AND FISSURE SEALANTS

A number of local factors which influence the success of pit and fissure sealants should be considered in future studies. One of these factors may include the fissure morphology of the tooth considered for sealant. Generally, shallow fissures were more readily sealed than deep narrow fissures. Penetration of the sealant material to the base of the fissure may occur more frequently in the shallow fissures. Significantly deep, narrow fissures may be related to the inability of the etchant material to flow into the depths of these narrow fissures.

Another factor influencing the success of pit and fissure sealants is isolation of the tooth to be sealed. Isolation is used to prevent moisture contamination of etched surfaces. Contamination by moisture was cited as one of the leading causes of bond failure between the sealant material and the enamel surfaces. Moisture contamination was not a factor in our in vitro study, because our specimen was treated on the laboratory bench.
Clinically, there may be significant differences in penetration and microleakage within our material and adhesive system in the face of moisture contamination.

Thermocycling was used in our study as a method to increase debonding of the material and the tooth and consequently to induce microleakage in a short time. However, the number of cycles, the immersion period, and the bath temperature has not been standardized to date.\(^{20}\) It should be considered a significant factor. The number of thermocyclings, the immersion period, and the bath temperature used in our study was based on Indiana University School of Dentistry standards and protocols consistently used for all studies requiring thermocycling.

Prophylaxis techniques used to clean the occlusal surfaces may influence bonding procedures. Clinically, occlusal surfaces are cleaned prior to the placement of a sealant. The use of pumice, a rotary prophy cup, mechanical debridement, and hydrogen peroxide has been recommended. Currently, the effect of hydrogen peroxide on the organic matrix is still under investigation. It has been suggested that the high concentration of hydrogen peroxide used in bleaching may adversely affect the bond strength of dental adhesive bonding agents.\(^{64,65}\) The 2.0-percent hydrogen peroxide used in our study is negligible when compared with the 30- to 35-percent used in bleaching. In addition, inability to remove all pumice within the fissures and fluoride in the prophy paste has been cited as also influencing the bonding of a sealant material. These factors should be investigated.

In the present study we used 150 extracted, caries-free, human third molars, selected for well-defined occlusal pits. Occlusal pits were considered well-defined if recognizable landmarks were present. For example, if small pinpoint depressions were located at the junction of developmental grooves or at the terminals of those grooves,
then they would be selected. Other landmarks referenced during the selection were the fossa, the sulcus and the developmental groove. Well-defined occlusal pits were based on a single evaluator, and judgment of well-defined occlusal pits will vary from one evaluator to another. Although there are variations among mandibular and maxillary third molars, no attempt was made to separate the two. They were randomly distributed into the six treatment groups, because it has been cited that 40 percent of the failed sealants on the mandibular first molar had failed because of caries in the buccal pits. Future studies should consider comparing the mandibular molars and the maxillary molars when investigating sealant material.

The hypothesis of this in vitro study was that there would be no significant difference in fissure penetration or microleakage between the conventional pit and fissure sealant or the flowable composite used as a pit and fissure sealant, regardless of the adhesive system used. The results of this study supported the hypothesis in that there was no significant difference in fissure penetration between the materials. The results, however, did demonstrate that there was significant difference in microleakage when the all-in-one adhesive system was used. Further research is indicated to verify the efficacy of convenient bonding procedures with fewer clinical steps.

The goals of this study were achieved. However, there are many other factors that may contribute to a pit and fissure sealant’s success that we did not simulate in our in vitro study and should be further investigated.
The concept of using a pit and fissure sealant material to prevent dental caries has been well established in dental research. Effectiveness of a pit and fissure sealant material is limited to its ability to remain bonded to the occlusal surfaces. Adding a dentin-bonding agent between the etched enamel and the sealant material has been demonstrated as a way of optimizing bond strength in the face of moisture and salivary contamination. Adaptation penetration and microleakage resistance of a pit and fissure sealant material ensures a physical barrier to the impaction of food and microorganisms into the sealed surfaces.

The purpose of this study was to determine if there is a difference in fissure penetration or microleakage between a conventional pit and fissure sealant or a flowable composite when used as a pit and fissure sealant, while using three different adhesive systems.

Delton Direct Delivery System (Dentsply), an opaque, light cured pit and fissure sealant was used. The commercially available flowable composite, Revelation Formula 2 (Kerr) was selected for this study. The three clinically used adhesive systems were: a conventional phosphoric acid etching and bonding application; Opti-bond Solo Plus (Kerr), a single bottle system; and Prosapt Lo-Pop (JM RSPE), an all-in-one primer adhesive. One hundred fifty extracted cavity-free, human third molars, selected for well-defined occlusal pits, were randomly divided into six treatment groups. Fissure penetration and microleakage was examined after immersion of the treated teeth in 5.0 percent methylene blue solution for 18 hours; the teeth were removed and thoroughly
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cleaned. Mesial and distal flat-ground sections were obtained and examined at X20; microleakage was recorded as either present or absent, and penetration was recorded as either complete or incomplete. If either one of the responses from the same tooth showed incomplete penetration, the response was treated as incomplete penetration. If either one of the responses from the same tooth presented microleakage, the response was treated as presenting microleakage.

The hypothesis of this thesis was that there would be no significant difference in fissure penetration or microleakage between the conventional pit and fissure sealant or the flowable composite, regardless of the adhesive system used. The results of this study supported the hypothesis in that there was no significant difference in fissure penetration between the materials. The results, however, did demonstrate that there was significant difference in microleakage between the three different adhesive systems used.

Within the parameters of this in vitro study it is concluded that:

1. There was no significant difference in fissure penetration between the conventional pit and fissure sealant or the flowable composite.

2. Microleakage was present in both the pit and fissure sealant and flowable composite regardless of the adhesive system used.

3. Enamel conditioning with the total-etch adhesive system or single-bottle adhesive system provided consistently better microleakage resistance compared with the use of the all-in-one adhesive system.

4. The all-in-one adhesive system demonstrated the most microleakage regardless of the material used for the pit and fissure sealant.


Tay FR, Pashley DH. Aggressiveness of contemporary self-etching systems. (Pt 1). Depth of penetration beyond dentin smear layers. Dent Mater 2001; 17:296-308


ABSTRACT

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The concept of using a pit and fissure sealant material to prevent dental caries have been well established in dental research. Effectiveness of a pit and fissure sealant material is limited to its ability to remain bonded to the occlusal surfaces. Adding a dentin-bonding agent between the occlusal enamel and the sealant material has been demonstrated as a way of optimizing bond strength in the face of moisture and solvency contamination. The purpose of this study was to examine if there was a difference in fissure penetration or microleakage between a conventional pit and fissure sealant or a
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The interaction between the material and the adhesive system was non-significant based on the logistic regression model for the penetration and the microleakage, so that only the main effects of material and adhesive system were included in the final model.

Enamel conditioning with the total-etch and single-bottle adhesive system provided consistently microleakage resistance when compared with the use of the all-in-one bonding system.

The all-in-one adhesive system demonstrated the most microleakage regardless of the material used for the pit and fissure sealant.

The hypothesis of this thesis was that there would be no significant difference in fissure penetration or microleakage between the conventional pit and fissure sealant or
the flowable composite, regardless of the adhesive system used. The results of this study support the hypothesis that there was no significant difference in fissure penetration between the materials. The result did demonstrate that there was significant difference in microleakage between the three different adhesive systems used.
CURRICULUM VITAE
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