ENAMEL CONDITIONING EFFECT ON PENETRATION AND MICROLEAKAGE OF GLASS IONOMER-BASED SEALANTS

by

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DEDICATION

This thesis is dedicated to those I love and whose images I always carry in my heart.

From the depth of my heart, I want to thank my loving parents, Lahib Rashid and Dr. Raad Ahmed, for their lifelong inspiration to be the best, for guiding me to the profession, for their endless love and support. You made me the person I am, and your example taught me that in this world, there is nothing impossible that parents cannot do for their children’s future.

To my brothers, Mustafa and Belal, thank you very much for your love and support; I am so proud to see your success. I know that one day you will be ones among the people that make a difference in this world and that all your dreams will come true.

To my amazing wife, Sura, and to my lovely daughter, Mariam: My deepest love and gratitude for all your help, care, patience, and encouragement during this journey. Thank you very much for all the happiness and joy you bring to my life and for making my career and this achievement possible.

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TABLE OF CONTENTS

LIST OF ILLUSTRATIONS

INTRODUCTION

Pit and fissure sealants can be used effectively as part of a comprehensive approach to caries prevention.[1] In 1955 Buonocore[2] predicted the ability to prevent caries by sealing pits and fissures with a bonded resin material. Later, in 1967 Cueto and Buonocore[3] published the first clinical study on pit and fissure sealants. Since then, there have been numerous reports documenting the efficacy of pit and fissure sealants.[1]

Most sealants available in the marketplace are resin-based. The preventive effects of this type of sealant are maintained as long as the material remains bonded to the dental surface.[4] However, for adequate retention of resin-based sealants, at the time of placement, the enamel must be clean, free of salivary contamination, and dry because of the hydrophobic nature of most resin-based materials, which is sometimes clinically challenging.[5, 6]

Glass ionomer cements were introduced in 1974 by McLean and Wilson[7] as an alternative sealant material. Glass ionomer materials are more tolerant of minor moisture presence during placement than their resin-based counterpart.[1] Moreover, they release fluoride ions into adjacent enamel and absorb fluoride from other sources, such as fluoride toothpastes and mouth rinses and thereby act as a rechargeable, slow-releasing fluoride device.[8] In addition, concerns have been raised about the likelihood that estrogenic chemicals, in particular bisphenol-A (BPA), might be leached out of dental resin sealants.[9]

In clinical studies, glass ionomer sealants have shown low retention and correspondingly high microleakage rates compared with resin sealants,[7, 10-15] and this has limited their use. However, a recent systematic review[16] concluded that there is no evidence for either resin-based or glass ionomer superiority among sealant materials in preventing dentin caries development in pits and fissures over time.

The ability of fissure sealants to prevent caries is directly related to sealant retention.[17] We hypothesize that improving penetration of glass ionomer sealants would reduce the amount of sealant exposed to occlusal stresses that cause cohesive failure, and that retention would improve.

A number of studies have evaluated different techniques of enamel conditioning for pits and fissures sealants. *In vitro* studies have shown that etching for 60 seconds instead of etching for 15 to 20 seconds with phosphoric acid is more effective at etching intact fissures and promoting reduction of microleakage.[17-19] Moreover, it has been shown that low viscosity surfactant-containing phosphoric acid significantly increases the resin-based sealant penetration into deep fissures.[20] It also has been shown that phosphoric acid etching preparation prior to the application of glass ionomer (GI) sealant significantly reduces microleakage.[18] Recently, a new self-etch conditioner developed to be used with resin modified glass ionomer (RMGI) was introduced (GC America). This new, self-etch conditioner has never been tested in combination with RMGI-based sealant. This self-etch conditioner has shown comparable microtensile bond strength to that obtained with a 25-percent polyalkenoic acid conditioner and has the advantage of not having to be rinsed off following application.[21]

The purpose of this study was to evaluate the influence of different fissure-conditioning techniques on penetration and microleakage of GI and RMGI cements used as sealants. Enhancing penetration and reducing microleakage of glass ionomer-based sealants may improve their retention and subsequently increase their preventive ability.

The hypothesis for this study was that fissure conditioning with one or more of the techniques being studied significantly increases penetration and microleakage resistance of GI or RMGI cements used as a sealant. The null hypothesis was that fissure conditioning with one or more of these techniques has no effect on penetration or microleakage resistance of GI or RMGI used as a sealant.

REVIEW OF LITERATURE

Over the last decades, the prevalence and severity of dental caries have declined, and the pattern shows detection of fewer smooth surface lesions than occlusal lesions.[22, 23] Dental caries is a site-specific disease that manifests itself primarily in pits and fissures. Fissures develop at the border line of closely located enamel-formation centers,[24] which have complex anatomy, especially deep and narrow, and difficult to access for self-cleaning or cleaning with conventional methods like tooth brushing. The prevalence of occlusal caries accounts for 56 percent to 80 percent of the lesions in permanent teeth.[25-27] The morphology of pits and fissures has been reported to be one of the most important caries risk factors,[28] with the molars more frequently affected than premolars, and mandibular molars more frequently decayed and restored than molars in the maxilla.[29, 30] By filling the pits and fissures with a restorative material (i.e., sealants), a cleansable, smooth surface can be created that would have a reduced risk for caries development.

PIT AND FISSURE SEALANTS

The term sealant is used to describe a material introduced into occlusal pits and fissures of teeth at risk of dental caries to create a barrier that reduces the impact of food and microorganisms, which may contribute to the formation of dental caries.[31] In 1955 Buonocore,[2] in a classic *in vitro* study, predicted the ability of sealants to prevent caries by using 85-percent phosphoric acid to etch pits and fissures for 60 seconds followed by the application of resin material. Later, in 1967 Cueto[3] verified the effectiveness of acrylic resin sealants in prevention of dental caries in a clinical study. He demonstrated that acrylic resin has the capability to remain bonded to the tooth surface and that bonding depends on a clean enamel surface that has been etched to produce microporosities.[3] The first dental pit and fissure sealant, Nuva-Seal (L.D. Caulk) was introduced in 1971 along with its curing initiator and ultraviolet light source. In the same year, sealants got acceptance by the American Dental Association. In 1974, glass ionomer cement was introduced as an alternative to the resin-based sealant.[7] In 1976, 3M Dental Products introduced the first white colored sealant (Concise White Sealant) by adding titanium dioxide, and in 2001 color-changing sealants by 3M ESPE and Ivoclar Vivadent.[32]

Reports for more than three decades have documented the efficacy of pit and fissure sealants in reducing occlusal dental caries.[1] Moreover, two studies on the effect of dental sealants on bacterial levels in caries lesions, and on the effectiveness of sealants in managing carious lesions, concluded that sealants prevent progression of dental caries. These two sets of findings suggest that “when sealants are retained, and thus access to fermentable substrates is blocked, bacteria do not appear capable of exerting their cariogenic potential.”[33, 34] Nowadays, in addition to resin-based sealants, glass ionomer-based sealants are available.

RESIN-BASED PIT AND FISSURE SEALANTS

Resin-based dental sealants are generally based on bis-phenol A glycidyl dimethacrylate (Bis-GMA) or urethane with the addition of diluents such as triethylenglycol dimethacrylate (TEGDMA) and/ or 2-hydroxyethyl methacrylate (HEMA). A wide variety of resin sealants is available, from unfilled to partially filled and from clear to white or other colors. These materials are either chemically polymerized or initiated by visible light.[32]

Despite the presence of two hydroxyl groups, the Bis-GMA monomer is inadequately hydrophilic to compete with water for interaction with the enamel surface.[35] Water within the microscopic capillaries would prevent complete penetration of the acrylic resin. Contamination of the etched enamel surfaces with saliva prior to sealant application will also prevent proper bonding, because the micropores become occluded.[36] Therefore, moisture contamination of etched enamel during application of the sealant is the most frequently cited reason for sealant failure.

GLASS IONOMER-BASED PIT AND FISSURE SEALANTS

In 1968, Smith[37] introduced the first cement (polycarboxylate) that bonds chemically to the tooth tissue.[37] Further work by Wilson's team[38] resulted in the introduction of glass ionomer (glass polyalkenoate) cements, based essentially on the liquid in polycarboxylate cements. The basic bonding mechanism is ionic attraction between two carboxyl (COO^-) groups in the cement to calcium (Ca^{++}) in enamel and dentin.

The conventional glass ionomer cements are water-based materials.[38] Since these are brittle materials, attempts were made to enhance the physical properties by addition of either metal particles (silver or gold),[39] by a fusion process resulting in a cermet (ceramic-metal), or an admix (amalgam alloy particles by a simple addition).[40] Further modification of conventional GI cements took place in early 1990s by the addition of water-soluble resin,[41] to produce the resin-modified GI cement..

Glass ionomer materials are more tolerant to minor moisture presence when being placed than their resin-based counterparts.[1] In addition, they can release fluoride ions into adjacent enamel and absorb fluoride from other sources, such as fluoride toothpastes and mouth rinses, and therefore act as rechargeable, slow-releasing fluoride devices.[8]

Clinical situations in which glass ionomer might be a better sealant alternative include treatment of children whose primary molars have deeply pitted or fissured surfaces; where isolation may be difficult; treatment of permanent first or second molars that have not fully erupted, and situations where a transitional sealant may be considered before placement of a permanent resin sealant.[1, 42]

The ability of fissure sealants to prevent caries is directly related to sealant retention.[17] In numerous clinical studies, glass ionomer-based sealants have shown low retention and correspondingly high microleakage rates compared with resin-based sealants,[7, 10-15, 43] However, in three clinical studies[44-46] glass ionomer sealants prevented dentin lesion development significantly better than resin-based sealants with the difference in sealant retention between the two types of materials being minimal. In 1996, Simonsen[5] did a critical review of literature on glass ionomer sealants:

“An objective assessment of the presently available scientific literature on the use of glass ionomer materials as pit and fissure sealants is not encouraging in terms of retention, but appears somewhat more positive for caries prevention. At the time of this writing [1996], the published literature indicates that retention for resin-based sealants is better than for glass ionomer sealants, but differences in caries prevention remain equivocal.”

Komatsu et al.[47] showed that high caries reduction rates could be obtained if constant reapplication of glass ionomer sealants is conducted when sealant is lost. The retention rate was maintained by sealant reapplication over three years. The authors concluded that reapplication is an acceptable procedure that seems to improve caries reduction.

Seppa et al.[48] found that fissures sealed with glass ionomer are more resistant to demineralization than control fissures, even after complete GI sealant lost. This may be the result of the combined effect of fluoride released by glass ionomer and residual material in the bottom of the fissures.[48, 49]

RESIN-MODIFIED GLASS IONOMER-BASED SEALANTS

Other researchers have begun to look at other glass ionomer materials such as RMGI cements as a sealant option. RMGI cement as an occlusal sealant in a one-year clinical study appeared to wear markedly.[50] Three clinical studies showed low retention of RMGI cement used as sealants compared with resin-based sealants with the difference in caries increment being minimal.[50-52] Furthermore, two of those studies showed that RMGI cement sealants continued to darken over the study; many became slightly darker than the sealed teeth. The increased darkness of the cement may possibly reflect water uptake, primarily, and breakdown of unreacted monomers and chromogenic compounds.[53]

Furthermore, in a recent clinical study, Vitremer (RMGI cement) with normal powder/liquid proportion (1:1) showed better retention performance than Delton (resin-based sealant) with cotton rolls, with or without a bonding agent.[54] These results suggested that RMGI cement may be an efficient and promising alternative as a dental sealant.

Pereira et al.[55, 56] conducted two clinical studies to assess retention and caries prevention of Vitremer (RMGI) and Ketac-bond (conventional glass ionomer) used as dental sealants. These studies showed higher retention for Vitremer, and no dental caries was recorded during the 12 to 24 months for both experimental groups. Moreover, Vieira et al.[57] conducted a clinical study finding that the RMGI presented higher retention rates than conventional GI sealants.

Other studies have been conducted to evaluate microleakage associated with RMGI. De Rego and Araujo[58] investigated the microleakage associated with different types of pit and fissure sealants and found greater microleakage for RMGI. The authors attributed the result to the fact that the enamel was not etched and that this type of material has a resin component.[58] Pardi et al.[59] conducted an *in vitro* study evaluating the microleakage of resin-based sealant, flowable composite resin-based sealant, compomer-based sealant, and RMGI-based sealant placed after 37-percent phosphoric acid etching. They found that all types of fissure sealants had similar marginal sealing ability. Results from this study suggested that etching pits and fissures with phosphoric acid may help in reducing microleakage associated with RMGI.

In summary, GI and RMGI are promising sealant materials. Recently, systematic review compared the caries-preventive effect of resin-based and glass ionomer-based sealants. The conclusion of this review was that there is no evidence for either resin-based or glass ionomer-based sealant material superiority in preventing dental caries development in pits and fissures over time.[16]

ENAMEL CONDITIONING METHODS

Buonocore[2] originally used 85-percent phosphoric acid for 60 seconds for etching enamel. Nowadays, as a result of better understanding of the acid etching process, most commercial enamel etchants are at 30-percent to 40-percent (commonly 37-percent) concentrations of phosphoric acid with recommended etching time of 15 to 20 seconds.[60]

However, regarding dental sealants, longer etching time (60 seconds vs. 15 to 20 seconds) with phosphoric acid has been shown to be more effective in etching intact fissures, and promoting reduction of microleakage under high relative humidity.[19] The longer etching time of 60 seconds might allow the etchant to penetrate better in deep fissures and therefore etch the enamel surface more effectively.[17] Reducing the etching period with 37-percent phosphoric acid has been shown to increase the number and size of voids between the sealant and enamel surface, which result in poorer adaptation of sealants to the vertical walls.[17] Moreover, it has been shown that low-viscosity surfactant containing phosphoric acid etchants can penetrate better into deep fissures producing a more retentive and wettable surface, significantly increasing the resin sealant penetration into deep fissures.[20] It also has been shown that phosphoric acid etching preparation prior to the application of glass ionomer-based sealant significantly improved their retention rate.[18]

Recently, GC introduced a new self-etch conditioner developed to be used with RMGI. This self-etch adhesive is mainly composed of 4-MET, HEMA, water, ethanol and initiator.[21] When used on enamel and dentin, this self-etch conditioner has shown comparable microtensile bond strength to that obtained with a 25-percent polyalkenoic acid conditioner, with the added advantage of not having to be rinsed off following application.[21] Additional conditioning steps with phosphoric acid plus using self-etch conditioner have shown to increase the bond strength of RMGI material.[21, 61] The new self-etch conditioner when used with RMGI seems to be a promising alternative to conventional conditioning with polyacrylic acid.

SEALANT PENETRATION EVALUATION

Enhancing sealant penetration into fissures should improve sealant retention.[17] Complete penetration of sealants into the fissure system is hard to achieve, especially into deep and narrow fissures,[62] due to the phenomenon of close-end capillaries or isolated capillaries.[63] Sealant penetration may be influenced by the geometry of the pits and fissures, presence of debris, air entrapment, and certain properties of the sealants themselves.[64]

Several studies tried to improve dental-sealants penetration by modifying pit and fissure cleaning and conditioning techniques. Different methodologies have been used to assess *in-vitro* sealant penetration. A study recorded penetration as complete or incomplete regardless of fissure length or complexity.[65] Other studies assessed the presence or absence of unfilled areas below the sealant material or measured the proportion of unfilled to filled areas.[66, 67] Some studies used the percentage of sealant depth to evaluate sealant penetration.[68] While other studies depend on a ranked scale system to assess sealant penetration, like the one described by Hosoya et al.,[69] the scoring system was as follows: “0 = no penetration; 1 = penetration restricted to the outer half of the fissure; 2 = sealant penetrated into inner half of the fissure; 3 = sealant penetrated into almost all fissures but one minor failure of adaptation or penetration; 4 = complete adaptation and penetration into all fissures." In summary, no consensus exists on a better method to evaluate sealant penetration.

MICROLEAKAGE EVALUATION

Microleakage is described as the movement of oral fluids between the tooth and dental restoration's interface. The fluid may include bacteria and toxic substances that may affect the tooth structure and the pulp.[70] The term microleakage has been cited in the literature since at least 1912.[71]

It has been suggested that microleakage increases the development of caries lesions.[72,73] *In-vitro* microleakage studies can predict the marginal sealing ability of dental sealants.[74] Moreover, measurement of microleakage has been used to evaluate the effectiveness of different conditioning procedures for the retention of fissure sealants *in vitro.*[17, 18, 75, 76]

Microleakage can be determined by different methods; however, evaluation of the penetration of a dye is the most simple and widely used methodology. Various solutions and dyes with different concentrations have been used to study the problem, such as radioactive isotopes,[77] methylene blue dye,[78] basic fuchsin,[79] erythrosine,[80] silver nitrate,[81] alcohol gentian violet,[73] and rhodamine.[82]

Different methods have been used to assess dye penetration through the tooth-sealant interface, such as by measuring the percentage of dye penetration along the enamel-sealant interface.[76] However, most studies used a ranked scale to score dye penetration, such as the one described by Grande et al.[83]: 0 = no dye penetration; 1 = dye penetration restricted to superficial margins; 2 = dye penetration restricted to lateral interface, and 3 = extensive dye penetration to the bottom of the sealant.

AGING BY THERMOCYCLING

Thermocycling is a widely used thermal fatigue method to evaluate the durability of dental material bond to tooth structure.[84] This method tries to mimic the thermal changes that occur in the oral cavity caused by eating, drinking, and breathing.[85]

This type of test induces frequent contraction/expansion stresses at the tooth-material interface resulting from the thermal contraction/expansion coefficient of dental materials that are different from the thermal contraction/expansion of the tooth.[86] This may result in crack propagation along the bonded interface and subsequent gap formation. Gaps of different dimensions can be created to allow the passage of fluids in and out of the interface.[85] Therefore, this methodology may modify the adhesion between the dental materials and tooth surface, showing the influence of thermal expansion on bond-strength.[87]

Thermocycling regimes used in reported studies differ with respect to the number of cycles, temperature, and dwell time. The number of cycles is frequently arbitrarily chosen. ISO standard (ISO TR 11450) proposed a regimen of 500 cycles.[88] In reported studies, cycling numbers range from 100 to 50,000 cycles; temperature extremes range from 4°C to 15°C in cold bath, and up to 45°C to 60°C in hot bath, while dwell time is usually 15 seconds, 30 seconds or 60 seconds.[89] It is anticipated that approximately 10,000 cycles correspond to one year of clinical function. This estimate is based on the hypothesis that such cycles may happen 20 to 50 times per day.[85]

METHODS AND MATERIALS

Human molars (IRB #0306-64) free from obvious caries lesions, morphological defects, restorations, and with deep pits and fissures that are typically indicated for sealant placement were selected. Teeth were kept in 0.1-percent thymol solution since extraction. All teeth had fully developed roots to homogenize the sample in relation to enamel maturation.

Teeth were randomly distributed into nine experimental groups of 15 teeth each. All teeth roots were cleaned with periodontal curettes. Occlusal surfaces were cleaned with a disposable prophylaxis angle with taper brush at low speed (5,000 RPM). The brush was immersed in water before any occlusal surface was brushed for 10 seconds. Brushed surfaces were flushed with an air-water spray for five seconds and lightly dried.

The enamel in the area of the pits and fissures was treated with different techniques and sealed with either resin-based sealant (Delton, Dentsply International Inc. York, PA, Lot No. 080428) or RMGI cement (Vitremer, 3M-ESPE Dental Products, St. Paul, MN. Powder lot No. 8AT, liquid lot No. 8HT) or GI sealant (GC Fuji triage-white, GC America Inc., Alsip, IL, Lot No. 0803251). Table I presents the different treatment groups.

Group A served as the control. Occlusal fissures were conventionally etched with 35-percent phosphoric acid gel etchant (Ultradent Products, South Jordan, UT) for 15 to 20 seconds, flushed with an air-water spray, and dried. Resin-based fissure sealant (Delton) was applied. Immediately after placement, each sealant was light cured (Coltolux 3, model No. C-7910, Coltene/ Whaledent Inc., Mahwah, NJ) for 20 seconds.

Group B served as the RMGI control. Occlusal fissures were conventionally conditioned with 25-percent polyacrylic acid (Ketac-conditioner, 3M-ESPE, Seefeld, Germany. Lot No. #258803) for 15 seconds, flushed with an air-water spray, and dried. RMGI cement (Vitremer) was mixed manually (hand-spatulated) using a standard powder/liquid proportion (1:1). Then, sealant material was delivered to the fissures using an intraoral delivery tip; a Q-tip dampened with Vitremer liquid was used to tamp cement into fissures and remove excess. Immediately after placement, each sealant was light cured for 40 seconds.

Group C: Occlusal fissures were etched with 35-percent phosphoric acid gel for 60 seconds, flushed with an air-water spray and dried. RMGI cement (Vitremer) was applied as a sealant, following the same steps of sealant placement as in group B.

Group D: Occlusal fissures were etched with low viscosity 35-percent phosphoric acid with a surfactant (SLS) for 60 seconds, flushed with an air-water spray and dried. RMGI cement (Vitremer) was applied as a sealant, following the same steps of sealant placement as in groups B and C.

Group E: Occlusal fissures were conditioned with self-etch conditioner (GC Self-conditioner; GC Corporation, Tokyo, Japan, Lot No. 0804081), and left undisturbed for 10 seconds. The surface was gently air-dried using compressed air for 5 seconds and ensured that the conditioned surface had a glossy appearance without water rinsing. RMGI cement (Vitremer) was applied as a sealant, following the same steps of sealant placement as in groups B, C, and D.

Group F: Occlusal fissures were etched with 35-percent phosphoric acid gel for 60 seconds, flushed with an air-water spray, and dried. Self-etch conditioner was applied and left undisturbed for 10 seconds. The surface was gently air-dried using compressed air for 5 seconds and ensured that the conditioned surface had a glossy appearance without water rinsing as done with group E. RMGI cement (Vitremer) was applied as a sealant, following the same steps of sealant placement as in groups B through E.

Group G served as the GI control. Occlusal fissures were conventionally conditioned with 25-percent polyacrylic acid for 15 seconds, flushed with an air-water spray, and dried. GI sealant (Fuji Triage White) capsule was triturated in a mixer for 10 seconds, capsule loaded into a capsule applier**,** and sealant applied into occlusal fissures.

Group H: Occlusal fissures were etched with 35-percent phosphoric acid gel for 60 seconds, flushed with an air-water spray, and dried. GI sealant (Fuji Triage White) was applied, following the same steps of sealant placement as in group G.

Group I: Occlusal fissures were etched with low viscosity 35-percent phosphoric acid with a surfactant for 60 seconds, flushed with an air-water spray, and dried. GI sealant (Fuji Triage White) was applied, following the same steps of sealant placement as in groups G and H.

Low viscosity phosphoric acid with surfactant was prepared by adding 14.2 ml of distilled water to 10 ml of low viscosity 85-percent orthophosphoric acid to produce low viscosity 35-percent phosphoric acid. One mg of sodium lauryl sulfate (SLS) was then added into 10 ml of the prepared low viscosity 35-percent phosphoric acid. Afterwards, the solution was stirred at 60°C for 10 minutes to dilute the SLS into the solution. That ended with production of 10 ml of low viscosity phosphoric acid with 0.1-percent of SLS.

After 48 hours storage in 100-percent relative humidity at 37 °C, the restored teeth were subjected to artificial aging by thermocycling in water for 2,500 cycles between 7°C ± 2 °C and 48 °C ± 3 °C with a dwell time of 30 seconds. The specimens' apices were covered with compound wax. After that, the whole specimens including the covered apices were painted with 2 layers of acid-resistant varnish except for their occlusal surfaces. Teeth were placed in separate containers and then each tooth was immersed in 1-percent methylene blue dye at 37 °C for 4 hours.[90]

After thermocycling and dye penetration, the teeth were rinsed thoroughly under tap water and the roots were removed using a saw. Teeth crowns were glued to plastic rods and were sectioned in three to five slices per tooth, depending on tooth size, with a hard tissue microtome saw (Gillings-Hamco, Hamco Machines Inc., Rochester, NY) The path of microtome saw sectioning was marked by extra fine Sharpie pen on the occlusal surfaces before sealant placement to make sure that sectioning would be through deep pits and fissures.

Each section was examined under stereomicroscopy (Nikon SMZ1500, Nikon, Tokyo, Japan). Images were captured, digitized, and analyzed using Nikon ACT-1 (version 2.63, Nikon Corporation, Tokyo, Japan) and Image J (Version 1.41, National Institutes of Health, Bethesda, Maryland). A ranked scale method described by Grande et al.,[83] was used to measure microleakage (Figures 1-4). The rank is as follow: (0) no dye penetration; (1) dye penetration into the occlusal third of the enamel-sealant interface; (2) dye penetration into the middle third of the interface; and (3) dye penetration into the apical third of the interface.

The penetration of the material into the pits and fissures was expressed as a percentage of the total length of the fissure, as previously described by Bottenberg et al.[20] (Figure 5). The fissure depth was measured from the point where the width of the fissure orifice becomes smaller than 200 μm down to the bottom of the fissure. The penetration depth was measured from the same point down to the deepest edge of the sealing material.[68]

STATISTICAL ANALYSIS

Comparisons among the groups for differences in the sealant penetration into the fissure were performed using analysis of variance (ANOVA). The ANOVA included a random effect for tooth to account for multiple sections analyzed from each tooth. The ranks of the penetration measurements were used in the analysis. Comparisons among the groups for differences in microleakage scores were performed using generalized estimating equation (GEE) methodology applied to cumulative logistic regression. Cumulative logistic regression is a method for analyzing outcomes with a limited number of ordered levels, and the GEE model was necessary to account for multiple sections analyzed from each tooth. Pair-wise comparisons between groups were performed because the overall test for any difference among groups was significant.

RESULTS

SEALANT PENETRATION

Table II shows a summary of the data collected for the sealant penetration percentage mean for each group. Resin-based sealant showed significantly better penetration than GI- and RMGI-based sealants. Conditioning fissures with either 35-percent phosphoric acid with or without SLS did not enhance either GI- or RMGI-based sealant penetration. Conditioning fissures with self-etch conditioner enhanced the penetration of RMGI-based sealant (Tables II and IV, Figure 6)

MICROLEAKAGE

Table III presents the data collected for microleakage scores for each group. Resin-based sealant showed significantly less microleakage than both GI and RMGI sealants. GI sealant showed less microleakage than RMGI-based sealant.

Regarding GI groups, conditioning fissures with 35-percent phosphoric acid with SLS showed significantly less microleakage than the GI control. Conditioning with 35-percent phosphoric acid gel showed no significant reduction in microleakage.

Regarding RMGI groups, conditioning fissures with 35-percent phosphoric acid with SLS showed less microleakage (marginally significant, $p = 0.06$) than the RMGI control. The rest of the conditioning methods tested did not reduce microleakage (Tables III and IV, Figure 7).

FIGURES AND TABLES

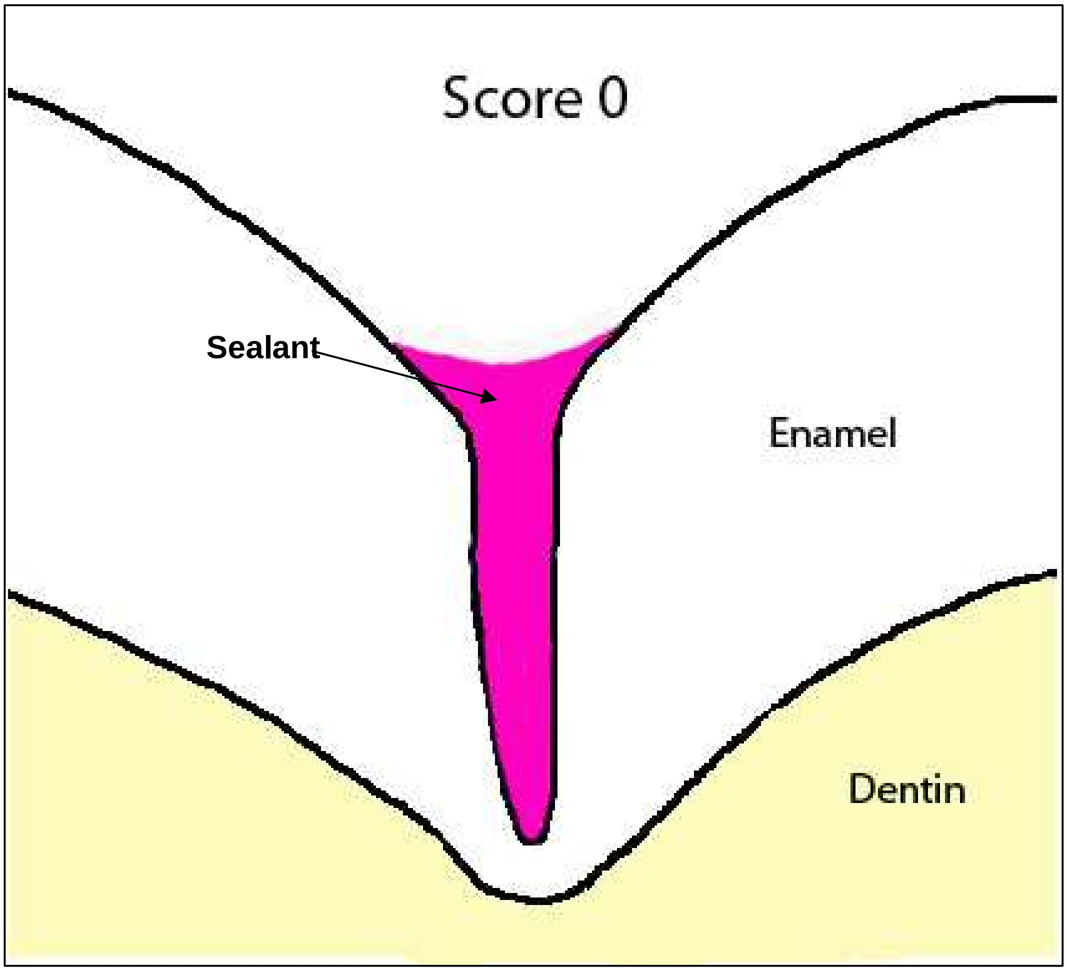

FIGURE 1. A diagram showing microleakage score 0, no dye penetration.

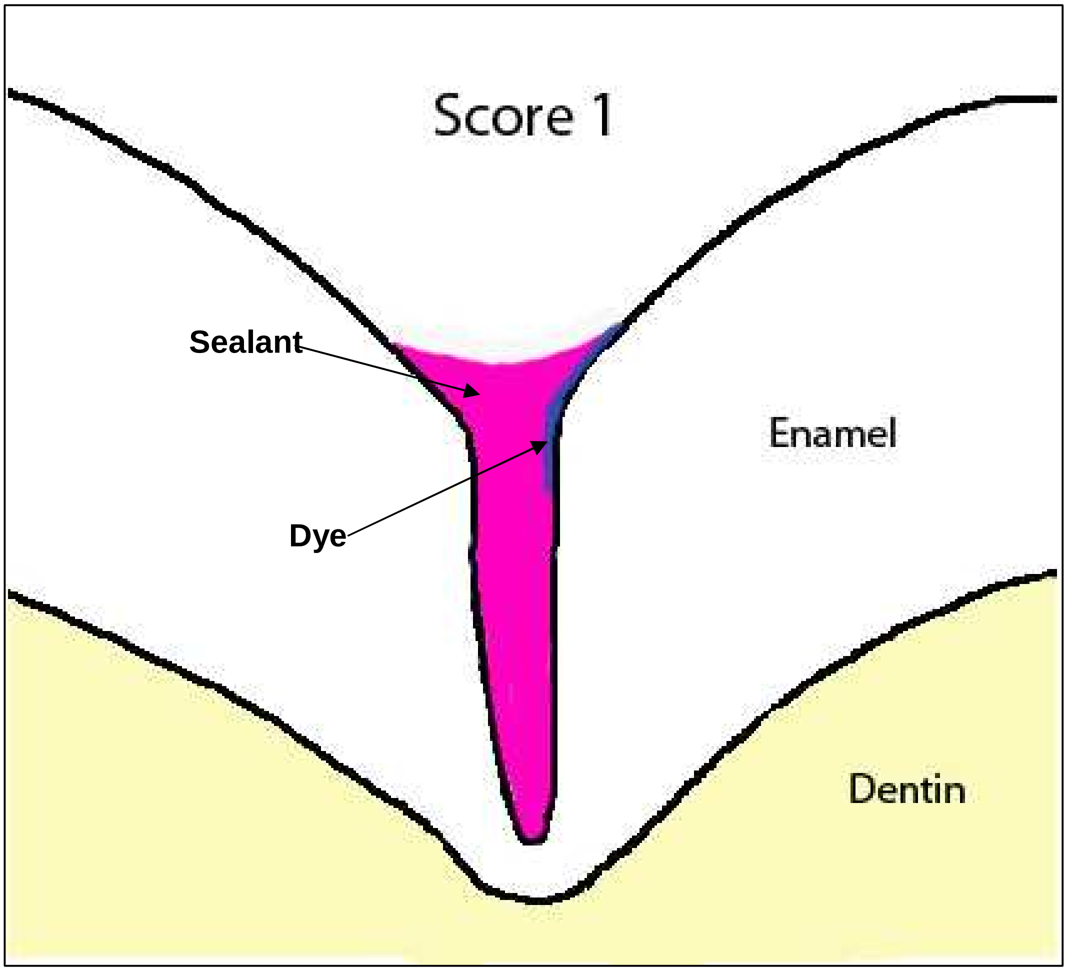

FIGURE 2. A diagram showing microleakage score 1, dye penetration into the occlusal third of the enamel-sealant interface.

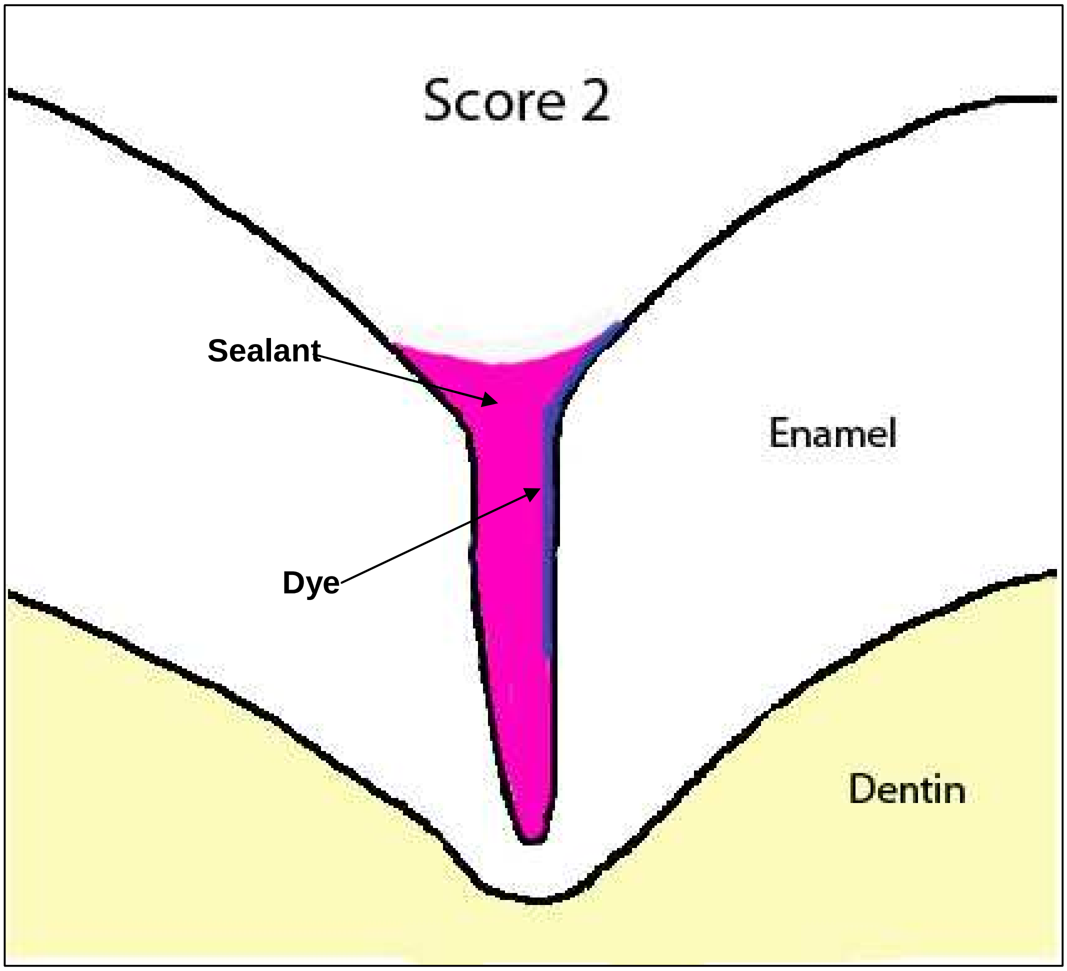

FIGURE 3. A diagram showing microleakage score 2, dye penetration into the middle third of the enamel-sealant interface.

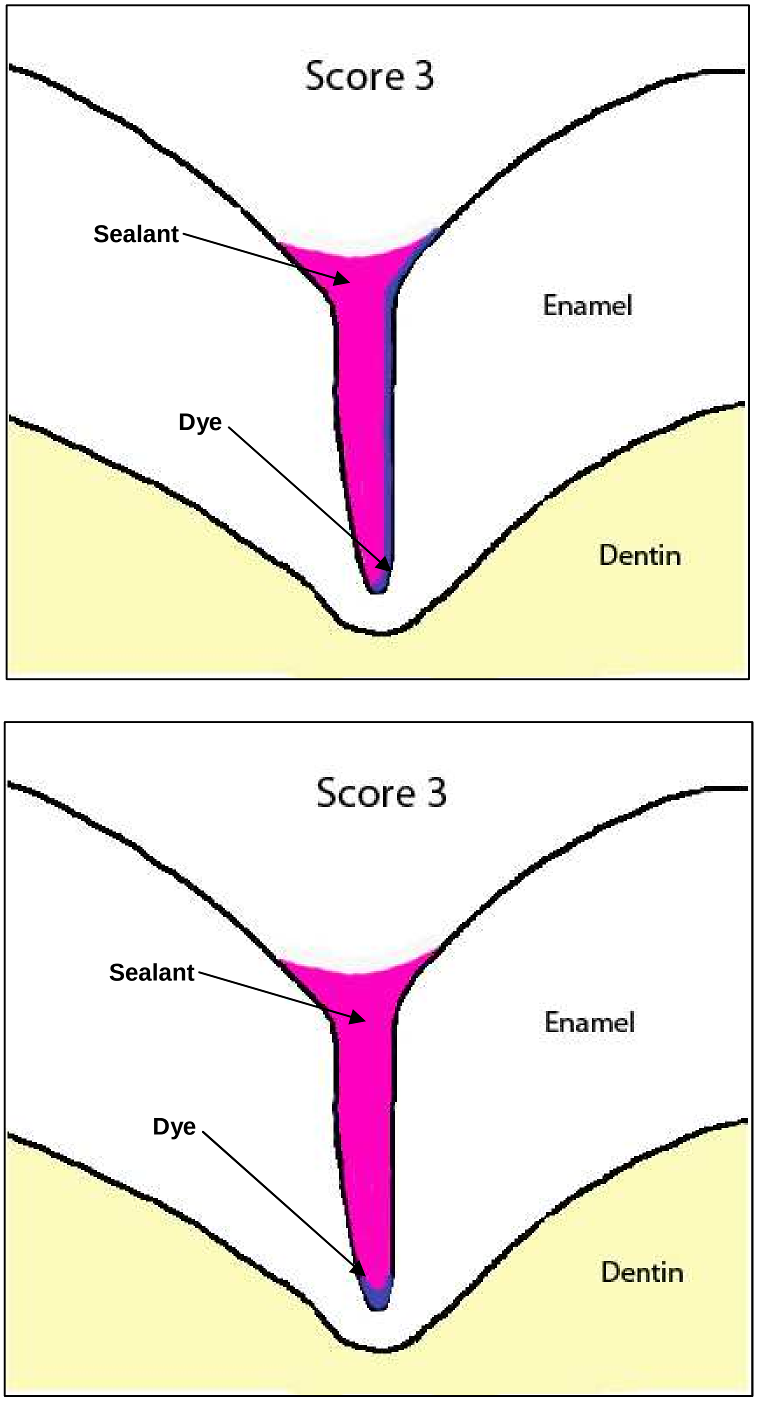

Figure 4. A diagram showing microleakage score 3, dye penetration into the apical third of the enamel-sealant interface.

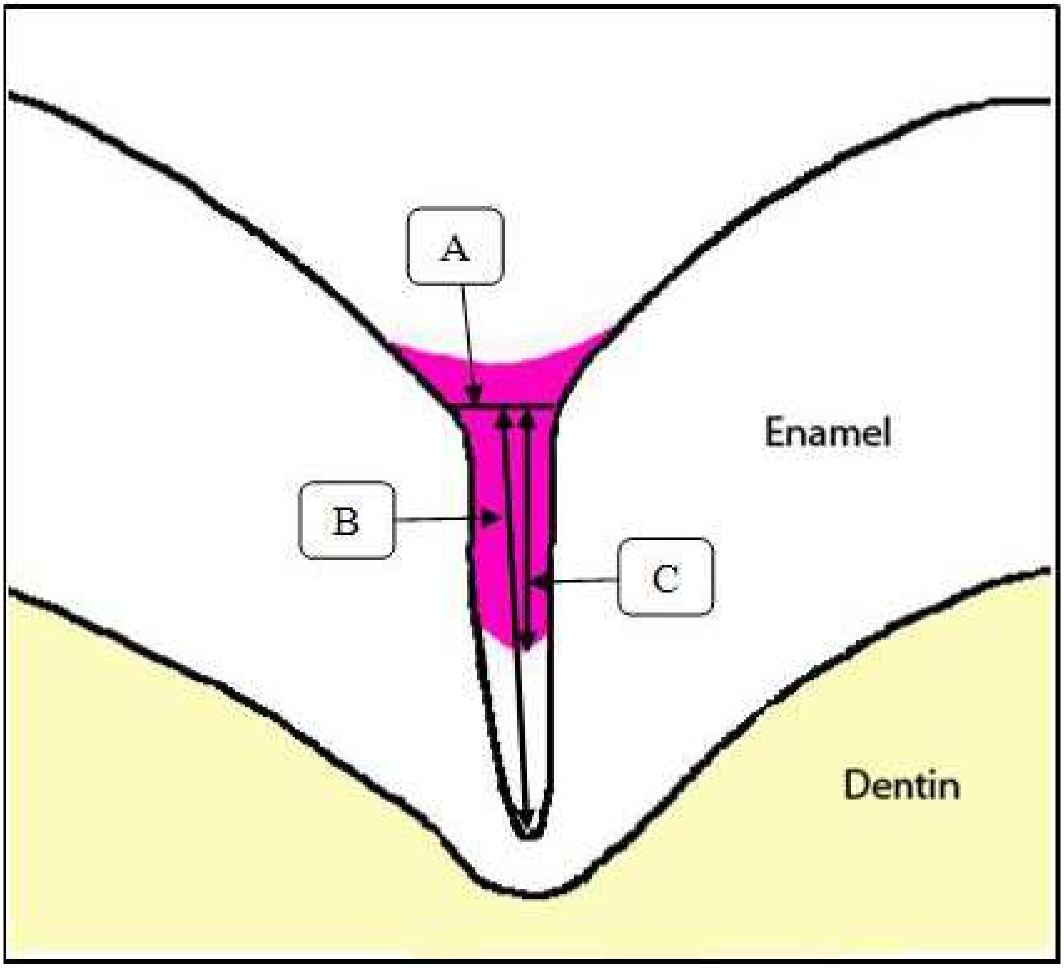

FIGURE 5. Method for quantifying penetration. A: Point where the width of the fissure orifice is 200 µm; B: Fissure depth from the width of 200 µm to the base of the fissure; C: Sealant penetration. Percent of sealant penetration was calculated using the formula AB/AC x 100.

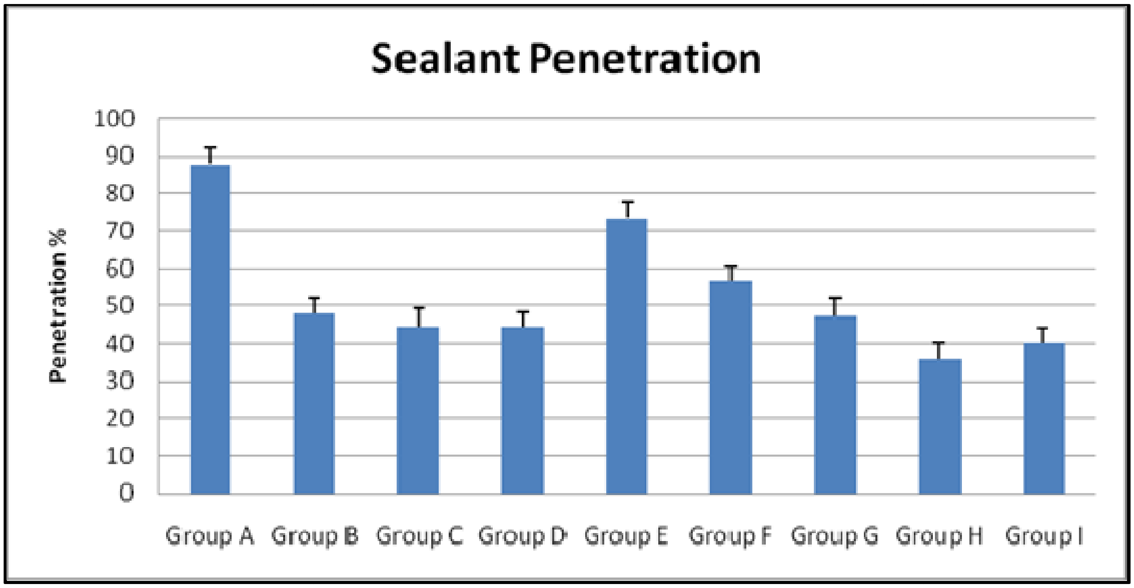

FIGURE 6. Average sealant penetration percentage of the different groups:
Group A: 35-percent phosphoric acid + Delton (Resin-based sealant);
Group B: 25-percent polyacrylic acid + Vitremer (RMGI-based sealant);
Group C: 35-percent phosphoric acid + Vitremer;
Group D: Low-viscosity 35-percent phosphoric acid w/surfactant + Vitremer;
Group E: Self-etch conditioner + Vitremer;
Group F: 35-percent phosphoric acid + self-etch conditioner + Vitremer;
Group G: 25-percent polyacrylic acid + Fuji Triage (GI-based sealant);
Group H: 35-percent phosphoric acid + Fuji Triage;
Group I: Low-viscosity 35-percent phosphoric acid w/surfactant + Fuji Triage.

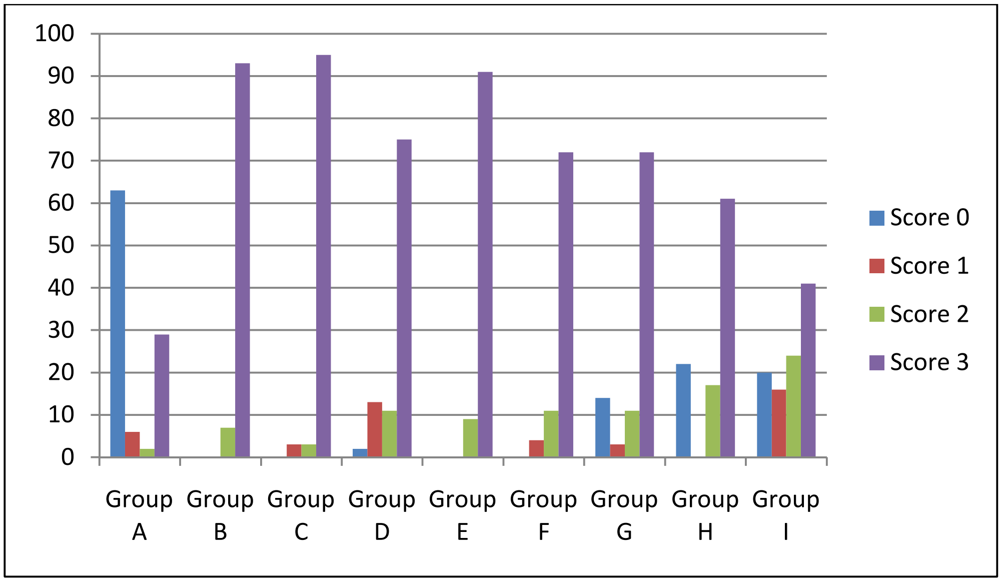

FIGURE 7. Average of microleakage scores of the different groups presented by percentage of specimens for each score.
Score 0: No dye penetration;
Score 1: Dye penetration into the occlusal third of the enamel-sealant interface;
Score 2: Dye penetration into the middle third of the interface;
Score 3: Dye penetration into the apical third of the interface.

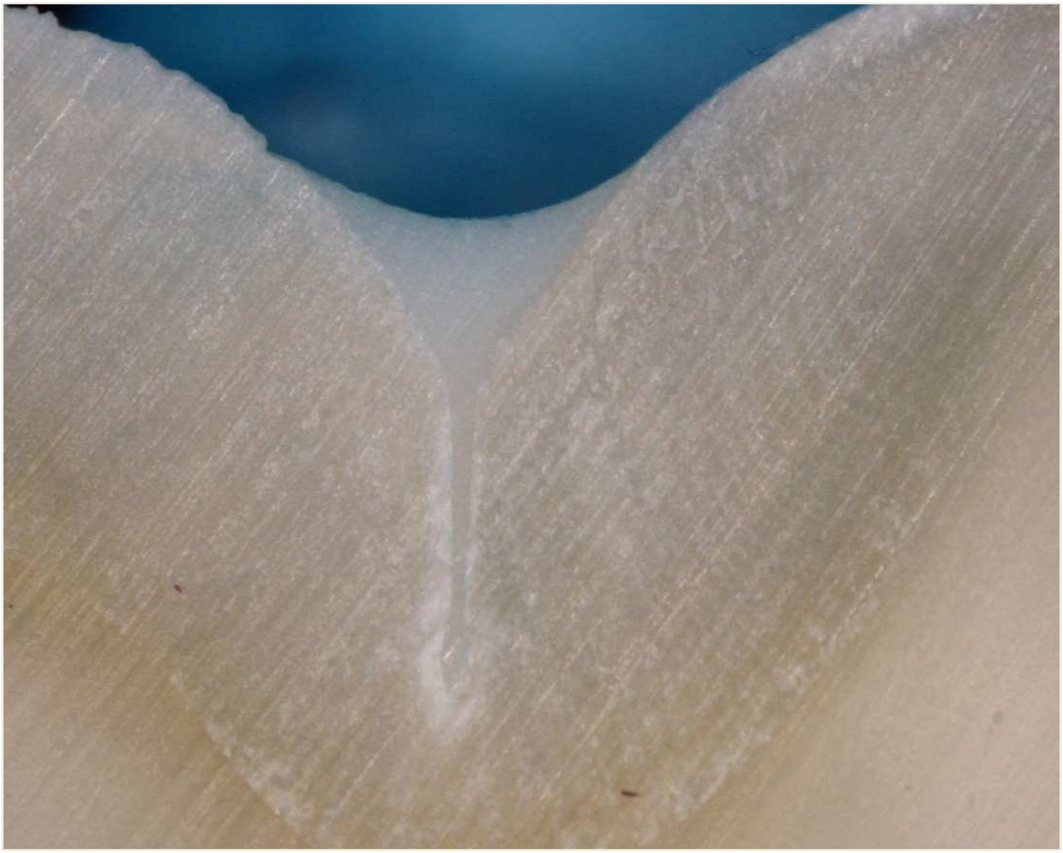

FIGURE 8. An example of complete fissure penetration of resin-based sealant and microleakage score 0.

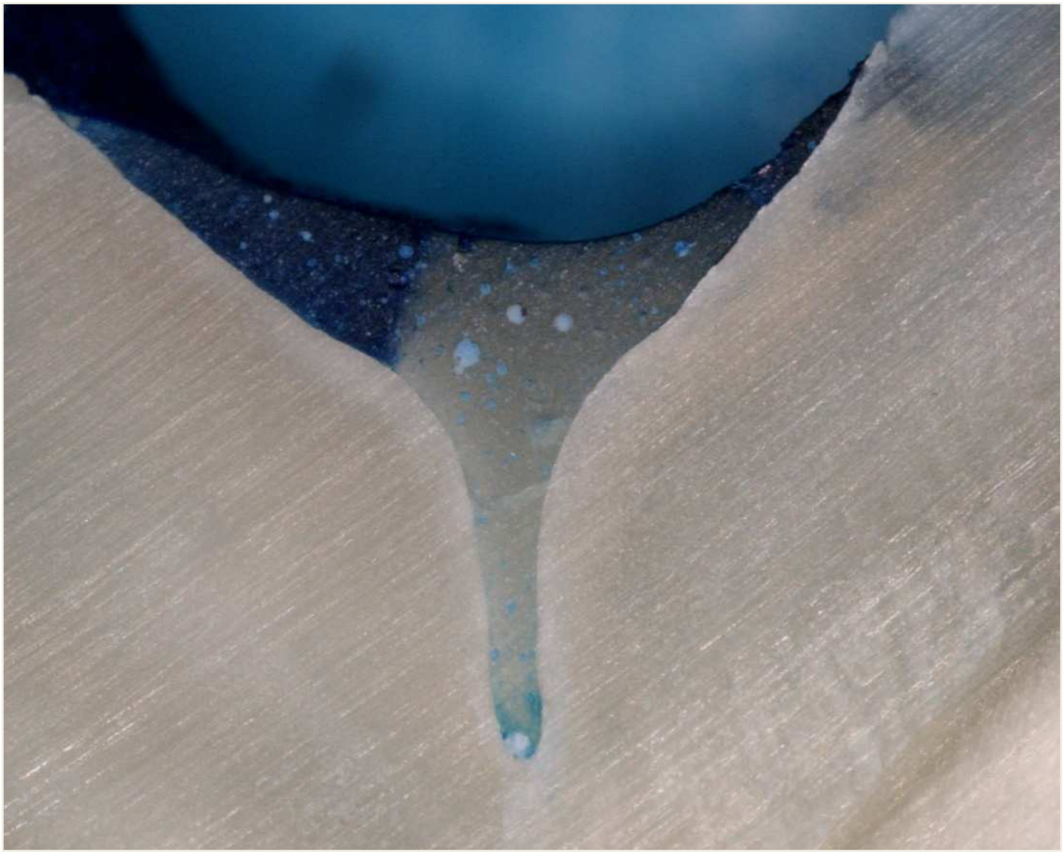

FIGURE 9. An example of RMGI-based sealant complete fissure penetration.

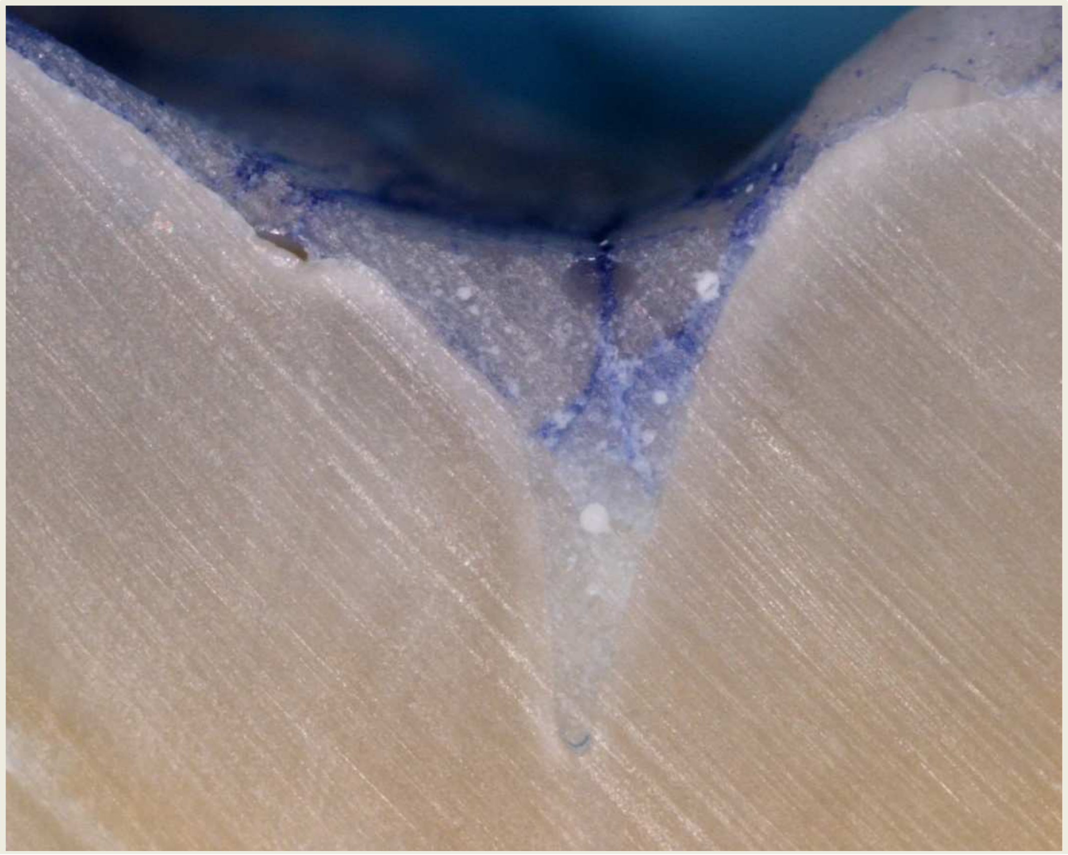

FIGURE 10. An example of GI sealant complete fissure penetration.

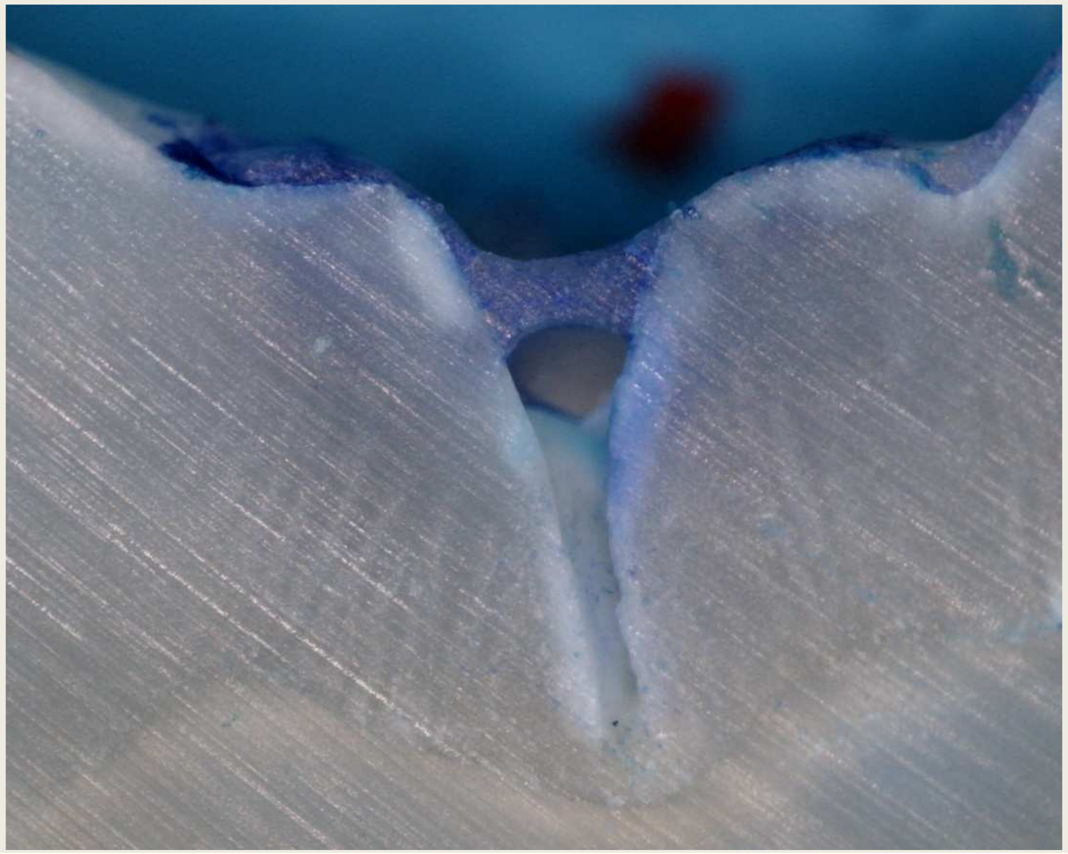

FIGURE 11. An example of GI sealant incomplete fissure penetration.

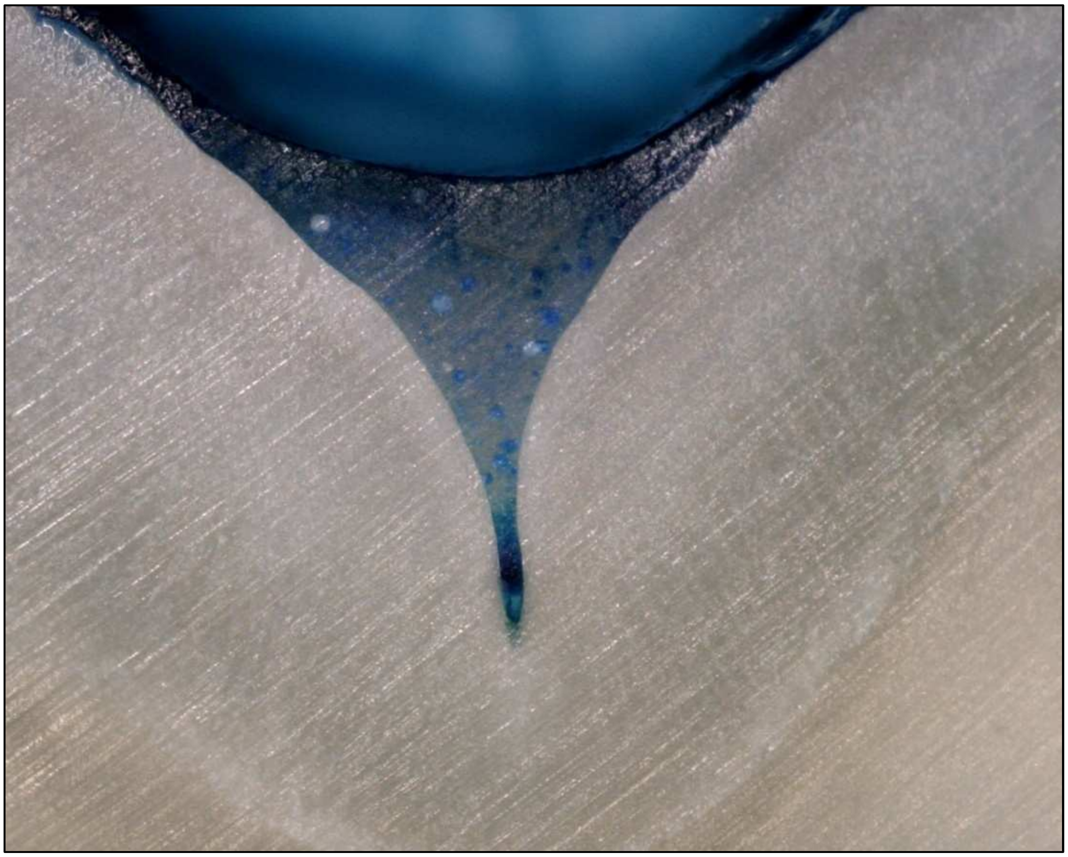

FIGURE 12. An example of dye penetration throughout the RMGI-based sealant and microleakage score 3.

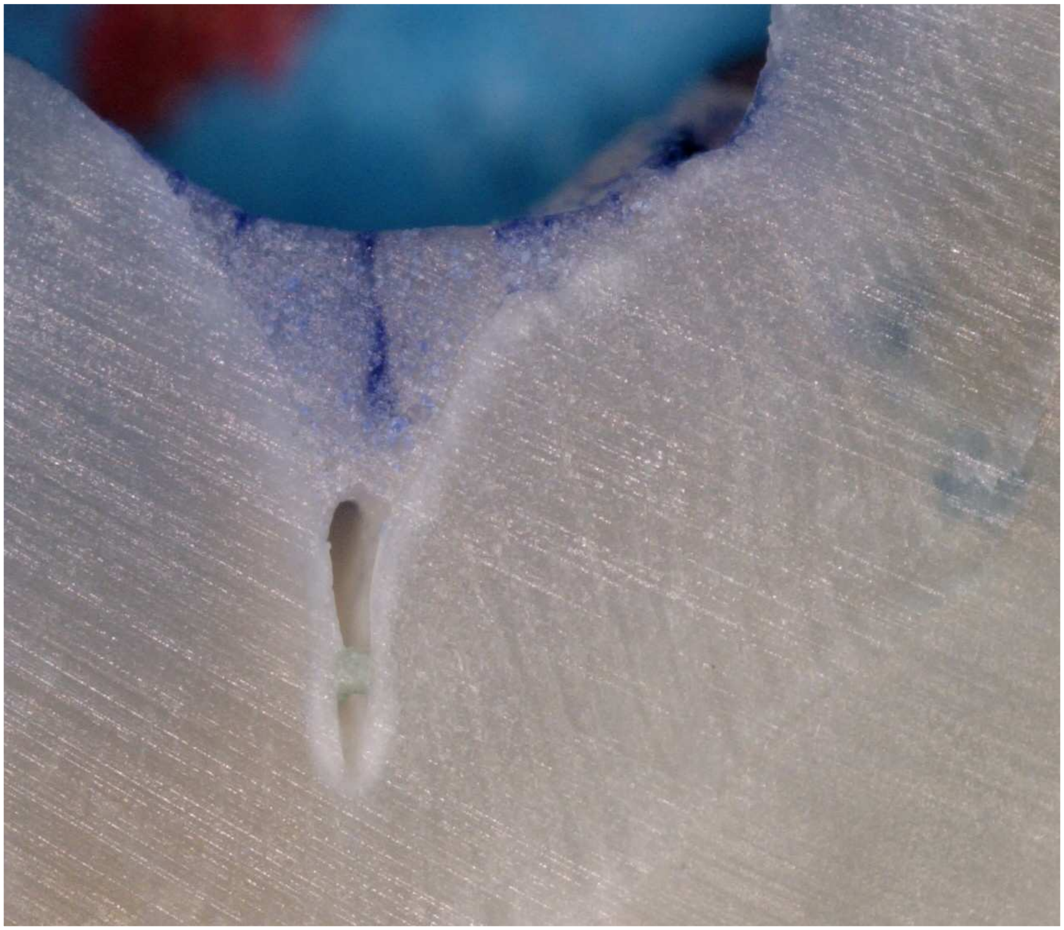

FIGURE 13. An example of incomplete fissure penetration of GI sealant and microleakage score 0.

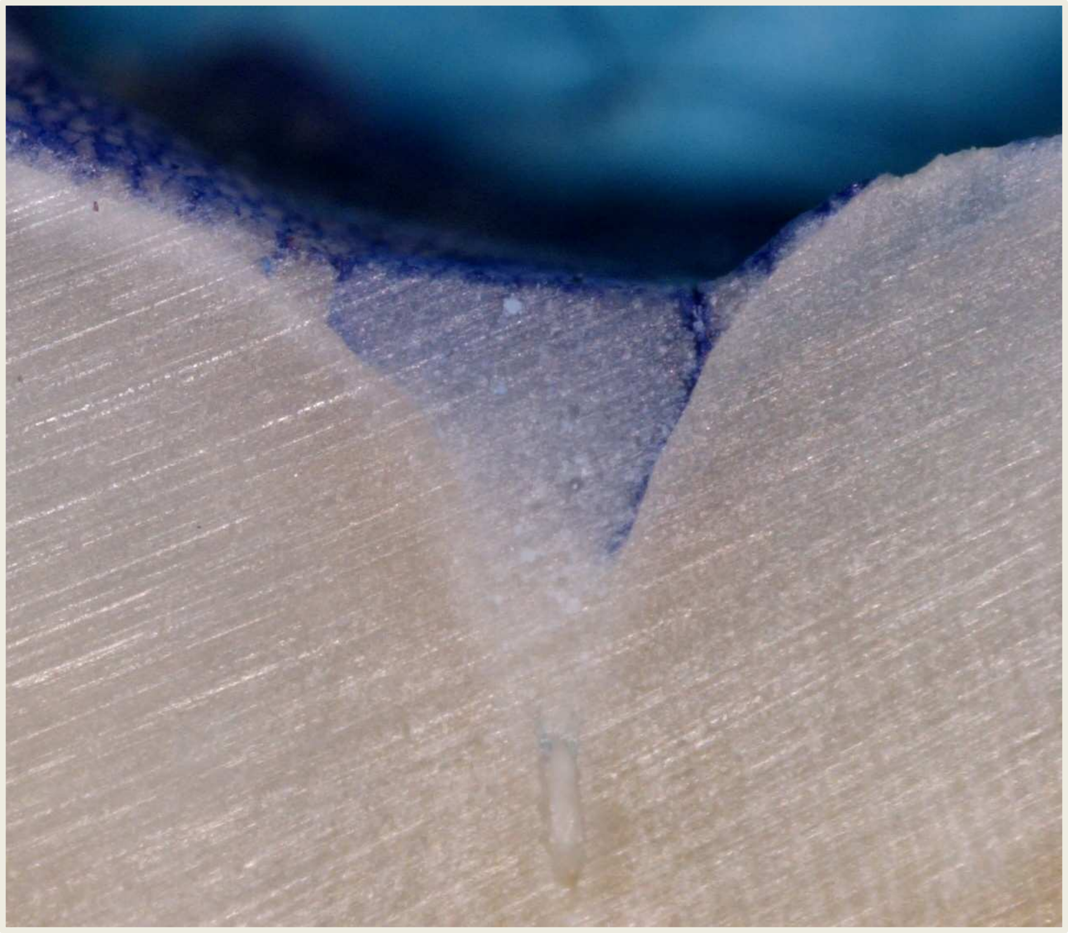

FIGURE 14. An example of microleakage score 2 associated with GI sealant.

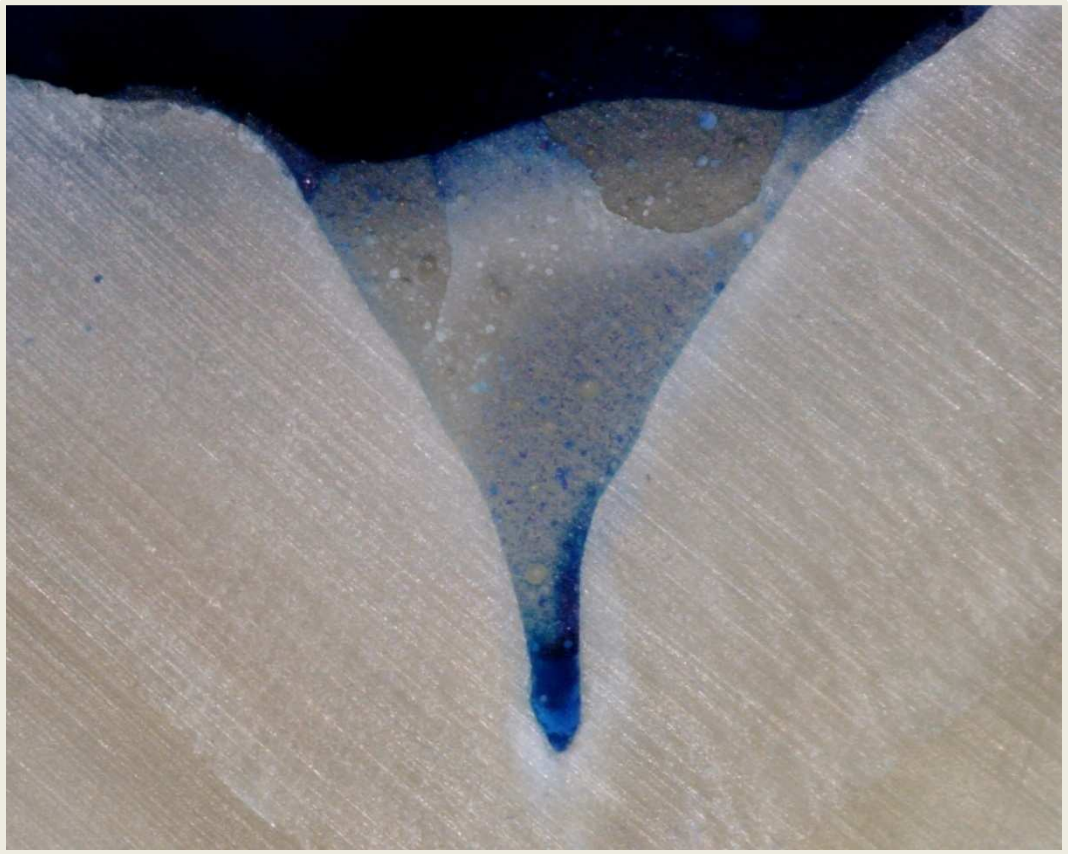

FIGURE 15. An example of microleakage score 3 of RMGI sealant. The dye penetrated to the apical third of the fissure.

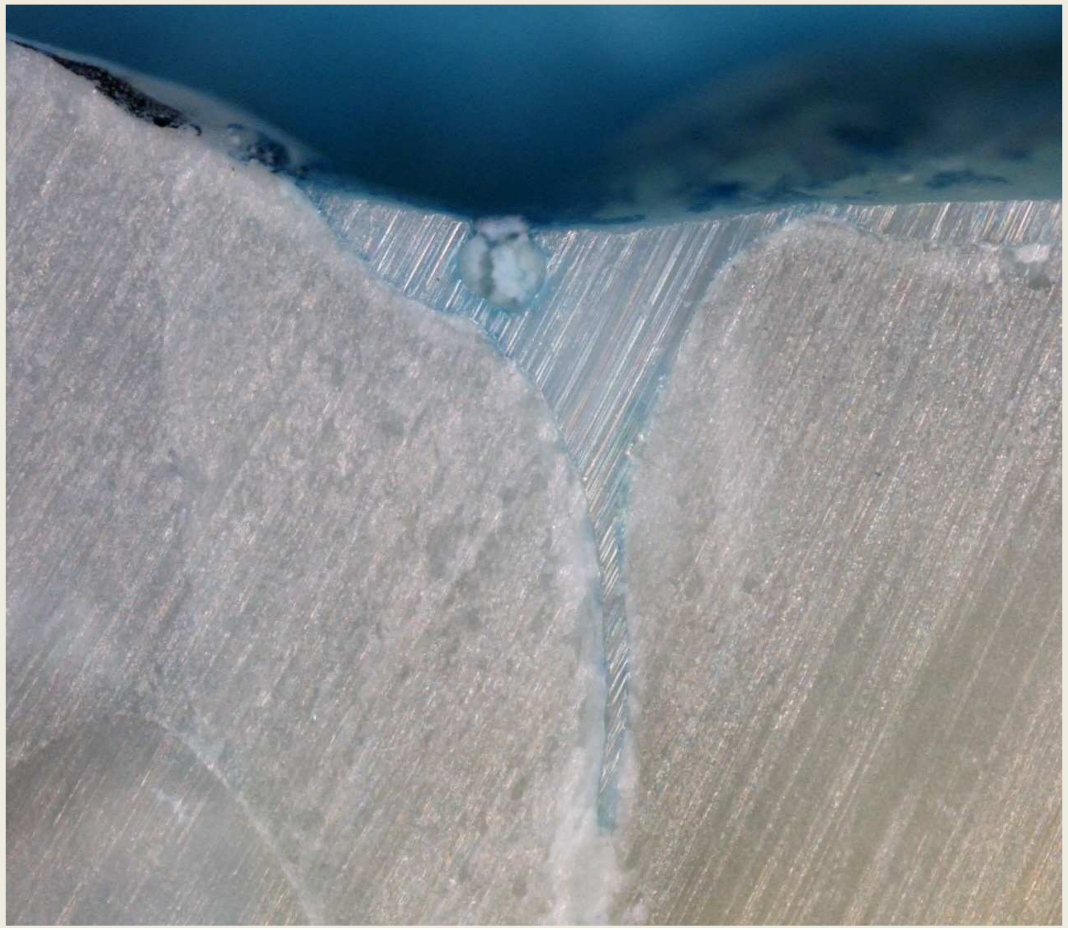

FIGURE 16. An example of microleakage score 3 associated with resin-based sealant.

TABLE I

Experimental groups*

Group	Sealant Type	Conditioning Method	Conditioning Time	
A	Resin	35-percent phosphoric acid	15-20 sec	control
B	RMGI	25-percent polyacrylic acid	15 sec	RMGI control
C	RMGI	35-percent phosphoric acid	60 sec	
D	RMGI	Low viscosity 35-percent phosphoric acid w/ surfactant	60 sec	
E	RMGI	Self-etch conditioner	10 sec	
F	RMGI	35-percent phosphoric acid + self-etch conditioner	60 sec 10 sec	
G	GI	25-percent polyacrylic acid	15 sec	GI control
H	GI	35-percent phosphoric acid	60 sec	
I	GI	Low-viscosity 35-percent phosphoric acid w/ surfactant	60 sec	

* GI: Glass ionomer.
RMGI: Resin modified glass ionomer.

TABLE II

Sealant penetration percentage

Group	Number of sections analyzed	Minimum penetration percentage	Maximum penetration percentage	Mean of penetration percentage (Standard Error)
A	49	14.5	100	87.9 (4.2)
B	41	0	100	47.8 (4.4)
C	37	0	100	44.7 (4.7)
D	56	0	100	44.5 (3.9)
E	55	0	100	73.6 (3.9)
F	46	0	100	56.8 (4.2)
G	36	0	100	47.3 (4.7)
H	41	0	100	35.7 (4.4)
I	51	0	100	40.2 (4.0)

TABLE III

Microleakage scores*

Microleakage, Number of sections (-percent)				
Group	Score 0	Score 1	Score 2	Score 3
A	31 (63)	3 (6)	1 (2)	14 (29)
B	0 (0)	0 (0)	3 (7)	38 (93)
C	0 (0)	1 (3)	1 (3)	35 (95)
D	1 (2)	7 (13)	6 (11)	42 (75)
E	0 (0)	0 (0)	5 (9)	50 (91)
F	0 (0)	2 (4)	5 (11)	39 (85)
G	5 (14)	1 (3)	4 (11)	26 (72)
H	9 (22)	0 (0)	7 (17)	25 (61)
I	10 (20)	8 (16)	12 (24)	21 (41)

* Score 0 (no dye penetration).
Score 1 (dye penetration into the occlusal third of the enamel-sealant interface).
Score 2 (dye penetration into the middle third of the interface).
Score 3 (dye penetration into the apical third of the interface).

Table IV

P-values for comparisons between groups

Comparison			Microleakage		Penetration	
A	vs.	B	<.0001	A < B	<.0001	A > B
A	vs.	C	<.0001	A < C	<.0001	A > C
A	vs.	D	<.0001	A < D	<.0001	A > D
A	vs.	E	<.0001	A < E	0.0194	A > E
A	vs.	F	<.0001	A < F	<.0001	A > F
A	vs.	G	0.0001	A < G	<.0001	A > G
A	vs.	H	0.0029	A < H	<.0001	A > H
A	vs.	I	0.0176	A < I	<.0001	A > I
B	vs.	C	0.7592		0.5703	
B	vs.	D	0.0631		0.6566	
B	vs.	E	0.7911		<.0001	B < E
B	vs.	F	0.3149		0.1568	
B	vs.	G	0.0256	B > G	0.7795	
B	vs.	H	0.0057	B > H	0.0263	B > H
B	vs.	I	0.0002	B > I	0.1509	
C	vs.	D	0.0176	C > D	0.8634	
C	vs.	E	0.5224		<.0001	C < E
C	vs.	F	0.1465		0.0514	
C	vs.	G	0.0051	C > G	0.7809	
C	vs.	H	0.0009	C > H	0.1079	
C	vs.	I	<.0001	C > I	0.4208	
D	vs.	E	0.0404	D < E	<.0001	D < E
D	vs.	F	0.2188		0.0497	D < F
D	vs.	G	0.6013		0.8952	
D	vs.	H	0.1402		0.0531	
D	vs.	I	0.0014	D > I	0.2817	
E	vs.	F	0.3545		0.0036	E > F
E	vs.	G	0.0095	E > G	<.0001	E > G
E	vs.	H	0.0014	E > H	<.0001	E > H
E	vs.	I	<.0001	E > I	<.0001	E > I
F	vs.	G	0.0699		0.0984	
F	vs.	H	0.0112	F > H	0.0003	F > H
F	vs.	I	<.0001	F > I	0.0034	F > I
G	vs.	H	0.2705		0.0600	
G	vs.	I	0.0040	G > I	0.2723	
H	vs.	I	0.2015		0.3630	

DISCUSSION

The purpose of this study was to evaluate the influence of different fissure conditioning techniques on penetration and microleakage of GI and RMGI cements used as sealants. Among all the conditioning techniques that were evaluated, only self-etch conditioner significantly enhanced the penetration of RMGI cement and only etching with low viscosity 35-percent phosphoric acid with surfactant (0.1-percent SLS) significantly reduced microleakage associated with GI sealant.

The self-etch conditioner used in this study was designed for placement of RMGI restorations. It has never been tested for conditioning of pits and fissures before using RMGI as the sealant. On the other hand, this self-etch conditioner had been evaluated for bond strength in bonding orthodontics brackets. Bishara and collaborators[91] found that brackets bonded with RMGI using the new self-conditioner had a shear bond strength comparable to brackets bonded with self-etch adhesive followed by composite resin or bonded with RMGI following etching with 10-percent polyacrylic acid. In addition, self-conditioner has the added benefit of not needing to be rinsed off and potentially decreasing technique sensitivity. The results of the present study showed that the use of self-etch conditioner increased significantly the penetration of RMGI-based sealant compared with the control (25-percent polyacrylic acid). This finding suggests that the self-etch conditioner may increase the wettability of the enamel in fissure system, which subsequently increases the penetration of RMGI cement. In contrast, etching the enamel pits and fissures with 35-percent phosphoric acid gel before using the self-etch conditioner did not enhance the penetration of the RMGI. While a previous *in-vitro* study[20] showed that etching with 35-percent low viscosity phosphoric acid with surfactant significantly increased resin-based sealant penetration into deep fissures, in the present study the conditioning with either 35-percent phosphoric acid gel or 35-percent low viscosity phosphoric acid with surfactant did not show any enhancement in penetration of RMGI and GI cements.

The results of this *in-vitro* study showed that in comparison to RMGI and GI cements, resin-based sealant has superior penetration into pits and fissures and penetrated the whole fissure more frequently. These results are in disagreement with the findings by Moore et al.,[65] who found that two types of light-cured glass ionomer cements (Fuji II LC and Vitremer) had superior fissure penetration than a resin-based sealant (Concise White). However, the penetration scoring system used in Moore's study was very different to the one used in this study. They only measured the number of specimens with complete fissure filling, which could be associated with penetration percentage, which was measured in our study.

Among all the conditioning techniques that were evaluated, only 35-percent low viscosity phosphoric acid with surfactant significantly reduced microleakage associated with GI sealant only. However, this reduction in microleakage was not significantly higher than that obtained by etching with 35-percent phosphoric acid gel. Etching with 35-percent phosphoric acid gel seemed to have a numerical trend for reduced microleakage when compared with the polyacrylic acid control, but the difference was not statistically significant ($p = 0.201$). These findings are in partial agreement with findings of a previous study done by Birkenfeld and Schulman[18] that showed etching pits and fissures with 37-percent phosphoric acid gel significantly decreased microleakage associated with GI sealants.

When evaluating microleakage in this *in-vitro* study, resin-based sealants showed significantly less microleakage than all teeth restored with GI- and RMGI-based sealants. These findings are conflicting with the findings of a previous study by Pardi et al.,[59] in which RMGI placed as sealant had sealing ability similar to the unfilled, self-cured resin-based sealant and flowable composite resin. All occlusal surfaces were conditioned by etching with 37-percent phosphoric acid gel, and there was no RMGI control.

GI and RMGI sealants showed extensive dye leakage throughout the material as well as at the interface between the material and the enamel (Figure 12). These results coincide with an *in-vivo* study using GI as a sealant. It was found that all specimens sealed with GI had extensive leakage with the dye penetrating throughout the material including the interface between cement and enamel. On the other hand, no leakage was found in the teeth sealed with composite resin. It was also found that the leakage was present even when the GI was fully retained.[11] GI- and RMGI-based sealants are porous materials, allowing microleakage through it as well as through the interface between the enamel and the sealants.[11] GI and RMGI porosity is necessary for easy leaching of fluoride ions to the surrounding tooth structure.[92, 93] In addition, GI cements require prolonged maturation time and should not be dehydrated within 6 months of placement.[94] Surface protection of glass ionomer cements during material setting and after placement is required to avoid desiccation and early solubility of the cement. [95] GI- or RMGI-based sealants used in this investigation were not protected with varnish or glaze-resin due to possible interference with the microleakage testing procedure. Preparation of specimens for this study may have led to some dehydration of the sealants, especially during application, because specimens' roots were covered with compound wax and nail varnish that required about 20 minutes under dry conditions for adequate setting of the materials. This drying period might have increased the microleakage throughout GI and RMGI sealants' material and through the material-tooth interface. These findings coincide with previous findings by Bouschlicher et al.[96] that showed unintentional desiccation of GI class 5 restorations prior to dye immersion had the effect of increasing the microleakage scores.

Attempting to reproduce clinical conditions, we used extracted human molars in this study to evaluate sealant penetration and microleakage. The *in-vitro* nature of the study allowed the control of multiple variables that could not be controlled under *in-vivo* conditions. On the other hand, these *in-vitro* conditions might exaggerate the level of penetration and reduce leakage that could be obtained clinically.

For the evaluation of microleakage, a ranked scale method described by Grande et al.[83] was used. This method has been used in several previous *in-vitro* studies assessing microleakage of different types of dental sealant.[59, 73, 97] *In-vitro* microleakage studies have the advantage of being able to predict the marginal sealing ability of sealants under controlled conditions.[74]

Sealant penetration methodology was adapted from two previously published methods.[20, 68] The method used allowed the control of several variables such as the starting measuring point, and the exclusion of certain irregular fissures. This modified method was expected to provide a more precise comparison of the penetration of the different groups.

In summary, the present findings suggest that the use of self-etch conditioner before placing RMGI-based sealants might enhance their penetration. They also suggest that the use of low-viscosity 35-percent phosphoric acid with surfactant before placing GI-based sealants might enhance their sealing properties. Controlled, randomized clinical trials are required to confirm these findings.

SUMMARY AND CONCLUSIONS

Sealing of pits and fissures is a common clinical procedure used to prevent occlusal caries. There are two types of dental sealants, resin-based and glass ionomer-based, the former the most commonly used. In clinical studies, resin-based sealants have shown superior retention and less microleakage rates than glass ionomer-based sealants.

However, available evidence does not support either resin-based or glass ionomer sealant material being superior to the other in preventing dental caries in pits and fissures over time. Glass ionomer-based sealants might be as effective at preventing caries as the resin-based sealants, and GIs more tolerant to minor moisture presence; therefore, they could be considered ideal materials for some clinical cases.

The retention of glass ionomer-based sealants might be improved by enhancing the penetration of the material into the fissure. Complete sealant penetration into pits and fissures reduces the possibility of fissure re-exposure upon the partial loss of sealant. It was hypothesized that modifying the conditioning technique might improve the penetration of glass ionomer-based sealants. It was also hypothesized that microleakage of glass ionomer-based sealants could be reduced by modifying the conditioning technique. Prevention of microleakage is considered to be an important function of fissure sealants.

The purpose of this study was to evaluate *in vitro* if microleakage associated with GI- and RMGI-based sealants can be reduced and sealant penetration can be enhanced by modifying the current conditioning methods. It was found that:

- Enamel conditioning with self-etch conditioner provided better RMGI-based sealant penetration.
- Enamel conditioning with low viscosity phosphoric acid with surfactant provided better GI sealant microleakage resistance.
- Resin-based sealant had significantly higher penetration than GI- and RMGI-based sealants.
- Resin-based sealant had significantly lower microleakage than GI- and RMGI-based sealants.

In summary, the present findings suggest that the use of self-etch conditioner before placing RMGI-based sealants enhances their penetration. The findings suggest that the use of low-viscosity 35-percent phosphoric acid with surfactant before placing GI-based sealants enhances their sealing properties. Controlled, randomized clinical trials are required to confirm the results of these findings.

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ABSTRACT

ENAMEL CONDITIONING EFFECT ON PENETRATION MICROLEAKAGE OF GLASS IONOMER-BASED SEALANTS

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While most sealants available are resin-based, glass ionomer-based cements can be used as sealants, with the advantage of being more tolerant to moisture during placement and of releasing fluoride. The objective of this study was to evaluate the influence of different fissure conditioning techniques on penetration and microleakage of glass ionomer (GI) and resin-modified glass ionomer cements (RMGI) used as sealants. Clinically sound extracted human molars were distributed into nine experimental groups (n = 15 each). Group 1 (control) was sealed with resin-based sealant (Delton) following clinically accepted techniques. Groups 2 through 6 were sealed with RMGI (Vitremer) after having the fissure conditioned with either polyacrylic acid (RMGI-control), 35-percent H_3PO_4, low viscosity 35-percent H_3PO_4 with a surfactant, self-etch conditioner, or 35-percent H_3PO_4 followed by self-etch conditioner. Groups 7 through 9 were sealed with GI sealant (Fuji

Triage) after having the fissures conditioned with either polyacrylic acid (GI-control), 35-percent H_3PO_4 or low viscosity 35-percent H_3PO_4 with a surfactant. After aging through thermocycling (2500 cycles), specimens were incubated in methylene blue for four hours and sectioned at multiple locations. Digital images were obtained using a digital stereomicroscope, and microleakage was determined by scoring the dye penetration along the enamel-sealant interface. The penetration of the material was determined by calculating the percentage of the total length of the fissure penetrated by the material. Results: The use of self etch-conditioner significantly increased RMGI penetration, while surface conditioning with 35-percent phosphoric acid with surfactant significantly decreased microleakage of GI. The resin-based sealant placed after 35-percent phosphoric acid surface conditioning showed the best penetration and the least level of microleakage. In conclusion, results from this study suggest that the placement of glass ionomer-based sealants can be enhanced by modifying current conditioning methods.

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