Comparative Evaluation for Microleakage between Hydrophilic Resin-Based Sealant and Sealant Containing S-PRG Filler With and Without Moisture Contamination: An In Vitro Study

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ABSTRACT

Objectives: The aims of this in-vitro study were to compare two types of sealant materials for microleakage and to evaluate the effects of saliva contamination on the microleakage of both types of sealants.

Methods: Forty-eight sound human third molars were randomly distributed into four groups: (1) UltraSeal XT hydro in dry condition; (2) UltraSeal XT hydro in contaminated condition; (3) BeautiSealant in dry condition; (4) BeautiSealant in contaminated condition. All teeth were subjected to thermocycling procedure followed by staining with 1% methylene blue dye. All teeth were embedded into resin then sectioned in buccolingual direction into three sections. The depth of dye penetration was evaluated using a stereomicroscope and was recorded according to the dye penetration score system (0-3). Data were analyzed with the Kruskal-Wallis and Mann-Whitney U tests.

Results: UltraSeal XT hydro showed significantly lower microleakage than BeautiSealant in both dry and contaminated conditions ($p<0.001$, $p=0.001$, respectively). There was no statistically significant difference in microleakage between UltraSeal XT hydro in dry and contaminated conditions. Also there was no statistically significant difference in microleakage between BeautiSealant in dry and contaminated conditions.

Conclusions: With the consideration to the limitations of an in-vitro study, we can conclude that UltraSeal XT hydro, in both dry and contaminated conditions, had significantly lower microleakage than BeautiSealant. There was not statistically significant evidence of a difference in microleakage between UltraSeal XT hydro in dry and contaminated conditions.

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Dental caries is the most common childhood disease. It is five times more common than asthma and seven times more common than allergic rhinitis.[1,2] It remains the greatest unmet health need among children of the United States.[3] Dental caries can have long term, negative consequences on a child's life. It can cause pain and infection which may impair a child's ability to eat, speak, socialize and may lead to poor overall health. It also adversely affects school attendance and performance.[4] Moreover, children with dental problems have more chance to develop oral health problems in their adulthood which can affect their quality of life and job prospects.[5] The good news is that states are performing many cost effective strategies to reduce dental caries, such as community water fluoridation.

Despite water fluoridation and despite the fact that the occlusal surface represents 12.5% of the total tooth surface,[6] 90% of dental caries in permanent molars in fluoridated communities are found to be pit and fissure caries.[7-9] This gives emphasis to the fact that fluoride treatment is more effective in preventing smooth surface caries than pit and fissure caries.[10] A survey conducted by the National Institute of Dental Research (NIDR) in 1986-1987 showed only 10% reduction of occlusal caries, compared to 60% reduction of proximal surfaces in a fluoridated community compared to the general population.[11] The anatomy of the fissure of posterior teeth makes it highly susceptible to plaque stagnation and microorganism adhesion.[12-14] Also the depth of the fissure that reaches the level of dentinoenamel junction makes the tooth more susceptible to caries.[15]

Attempts to prevent occlusal caries have been reported from the beginning of the 20th century. In 1924, Thaddeus Hyatt advocated prophylactic restorations in which amalgam restoration was placed in class I cavity preparation to cover all susceptible pits and fissures.[16] A more conservative approach was promoted by Bodecker in 1929. He advocated the prophylactic odontomy technique that aimed to convert the deep, retentive fissure to cleanable fissures by mechanical eradication.[17] In 1955, Buonocore started a new era of dentistry when he discovered a method for enamel preparation using weak acid. The first clinical value from Buonocore's study was the use of pit and fissure sealants as a method of caries prevention.[18,19] A fissure sealant is a type of preventative measure where material is placed in the pits and fissures of posterior teeth to inhibit the development or the progression of dental caries.[20] The sealing materials act as a physical barrier between fissure microorganisms and their source of nutrients.[2,19,21,22] Pit and fissure sealants have been considered the most effective method in preventing occlusal caries.[19,21,22]

Researchers were looking for an ideal material to be used to seal occlusal pits and fissures. Various types of materials have been produced to cover caries susceptible pits and fissures. The first sealant material introduced was cyanoacrylates, but it was unsuitable for oral use because of its bacterial degradation in the oral cavity.[23] In 1960s, BIS-GMA resin was introduced by reacting bispehnol A with glycidyl methacrylate, and found to be resistant to degradation and produce strong bond with etched enamel.[23] Since then, resin based fissure sealants became the most commonly used sealant.[24]

The preventive effects of resin based sealants depend mainly on the micro mechanical retention of the sealant material on the fissure.[25] Due to the hydrophobic nature of resin based sealants, the tooth must be dry and free of saliva contamination during placement of the sealant material to achieve the maximum retention, which can be difficult in some clinical situations.[26,27] Therefore, salivary contamination is considered one of the main causes of sealants failure.[28,29]

After the invention of Fluoroaluminium silicate glass ionomer in the 1970s, glass ionomer has been used as an alternative sealant material to the resin-based pit and fissure sealant.[30] In comparison with resin based sealants, glass ionomer sealants have lower retention and more microleakage than the resin based sealant.[31-34] But the main advantage of glass ionomer sealant materials over resin based sealants is the ability of glass ionomer sealant to release fluoride ions to adjacent enamel and recharges by absorbing fluoride from different sources such as fluoridated tooth paste and mouth rinses. Therefore the glass ionomer sealant becomes a slow releasing fluoride device.[35] Fluoride released from glass ionomer sealants has a protective effect by preventing demineralization in the adjacent tooth structure.[33] Furthermore, glass ionomer sealants are easier to place and are more tolerant to minor moisture presence during placement than resin based sealants.[30,36] The hydrophilic characteristic of the glass ionomer material makes it a better alternative to resin based sealants in situations when moisture control is difficult, such as placing sealants in young uncooperative children or placing sealants over partially erupted teeth. It also can be used as a transitional sealant before placement of a permanent resin based sealant.[36,37]

Resin based and glass ionomer based materials have been used as sealant materials for a long time. Regarding marginal microleakage, the glass ionomer sealant material has better results compared to resin based sealants when sealants are placed under contaminated conditions;[38] however, when placed in ideal conditions the glass ionomer sealant material has lower retention.[31] To overcome the retention and microleakage problems of glass ionomer sealant materials, some researchers investigated the use of resin modified glass ionomer as an alternative sealant material. Studies showed that resin modified glass ionomer fissure sealants still have lower retention rates than the resin based fissure sealant.[39,40] Another study showed that resin modified glass ionomer sealants wear markedly in a one year clinical trial.[41] In comparison with conventional glass ionomer sealant materials, studies showed that resin modified glass ionomer still has a higher retention rate than conventional glass ionomer.[42]

Despite the type of material used, pits and fissures sealants are still the most effective technique in caries prevention in occlusal surfaces. Studies have shown that sealant application causes 86% caries reduction after one year, 78.6% after 2 years and 58% after 4 years.[8,43,44] Despite these promising statistics, sealant effectiveness in caries prevention decreases with time. Studies have shown that caries rates have increased from 4% after the first year of sealant application, up to 31% after 7 years of sealant application.[8,45,46] The main cause for decreased sealants effectiveness is the lack of sealant retention which decreased from 92% after the first year to 71% after 3 years to 66% after 7 years.[8,45,46]

Saliva contamination has been found to be one of the most important factors affecting sealant retention. Studies show saliva contaminations after tooth preparation with acid etch. Subsequently, this prevents tag formation and decreases the bond strength of sealants and thereby decreases the mechanical retention.[26,36,47] The effectiveness of pit and fissure sealants in caries prevention depends mainly on sealant retention.[25] Saliva contamination increases microleakage and leads to sealant failure.[26,27] Microleakage is defined as the movement of fluids between the tooth and dental material interface, which may include bacteria and toxic substance that may affect the tooth structure and pulp.[48] Microleakage under dental restorations increases the development of dental caries.[49] Measurement of microleakage has been used in the literature to evaluate the marginal sealing ability of sealant materials.[1,50-54] Optimal moisture control should be obtained before sealant placement to minimize the chance of microleakage. Maintaining a dry field can prove challenging in certain clinical situations, especially with a partially erupted molar.[26,27] Researchers found that the greatest period of risk for caries is within 2-4 years of eruption of the tooth when the enamel is not yet fully matured.[55] A hydrophilic sealant material that still has good marginal sealing ability under contaminated condition would eliminate the need to postpone sealant placement on caries susceptible teeth when moisture control is difficult. Recently, two sealant materials have been produced to overcome retention problems that occur when a sealant is applied under contaminated condition. The first is BeautiSealant made by Shofu Inc. The second is UltraSeal XT hydro made by Ultradent Products, Inc.

BeautiSealant is a giomer, a material containing Shofu's proprietary Surface Pre-Reacted Glass (S-PRG filler particles). It is a resin-based fissure sealant that can be placed without need for phosphoric acid etches and rinse, eliminating these steps entirely. Instead, BeautiSealant requires the use of a self-etching primer that is significantly less acidic, helping preserve healthy tooth structure. S-PRG filler releases 6 ions: Fluoride, Sodium, Strontium, Aluminum, Silicate, and Borate, all with bioactive properties. Due to the giomer structure, BeautiSealant does not require water absorption following photo cure to release fluoride. The unique structure form of the giomer will supposedly protect the glass core from the damaging effects of moisture, allow ions release and recharge to take place, and improve long term durability.[56]

UltraSeal XT hydro is a 53% filled, light-cure, radiopaque, methacrylate-based, thixotropic resin sealant. It is a BPA-free hydrophilic resin based sealant, which the company claims will eliminate the need for use of a drying agent before application and ensure marginal retention.

Finding a hydrophilic sealant material with good marginal sealing ability would be an essential step to protect caries susceptible teeth even in instances where optimal moisture control is unachievable. One or both of these materials may have similar retention rates whether placed under dry or contaminated conditions. The purpose of this study was to compare the microleakage between the two new sealant materials, UltraSeal XT hydro and BeautiSealant, and to evaluate the microleakage under the effects of saliva contamination on both types of sealants.

SPECIFIC AIMS AND HYPOTHESIS

The aims of this study were to compare two types of sealant materials for microleakage, and to evaluate the effects of saliva contamination on the microleakage of both types of sealants.

Hypothesis 1: UltraSeal XT hydro has less microleakage than BeautiSealant in both dry and contaminated conditions.

Hypothesis 2: UltraSeal XT hydro and BeautiSealant have less microleakage when placed in dry conditions than in contaminated conditions.

LITERATURE REVIEW

A) OCCLUSAL PITS AND FISSURES

Dental caries is an infectious disease caused primarily by mutans streptococci.[57] The anatomy of occlusal pits and fissures provides an ecologic niche for *Streptococcus mutans* colonization.[12-15,58] Therefore, to inhibit bacterial growth, occlusal pits and fissures sealant should be placed.[58] *Streptococcus mutans* has a difficult time surviving below fissure sealants.[59] Studies found that sealed pits and fissures had less bacterial counts than unsealed pits and fissures.[60,61]

B) RESIN BASED FISSURE SEALANT

Resin based fissure sealants are based on the reaction between bispehnol A with glycidyl methacrylate (BIS-GMA).[23] BIS-GMA combines the desirable properties of both methacrylate and epoxy molecules. It is characterized by the rapid polymerization characteristic of methylmethacrylate and the minimal polymerization shrinkage property of epoxy resins.[23]

The hydrophobic nature of BIS-GMA makes it unable to compete with water for interaction with etched enamel.[62] Water prevents complete penetration of the acrylic resin and thereby decreases the mechanical retention.[26,36,47] Therefore, the placement of resin based sealants is considered technique sensitive. Efficient moisture control should be obtained during application to achieve the maximum retention, which can be difficult in some working environments.[26,27]

C) GLASS IONOMER FISSURE SEALANT

The glass ionomer material was invented in 1969 by Wilson and Kent.[63] It is made of calcium or strontium aluminofluorosilicate glass powder in association with a water soluble polymer.[63] In 1974, glass ionomer materials were introduced by McLean and Wilson as an alternative sealant material to the resin based pits and fissures sealant.[30] The main advantage of glass ionomer fissure sealant over resin based fissure sealant is the ability of glass ionomer material to release fluoride.[35] Moreover, the hydrophilic nature of the glass ionomer material makes it compatible with minor moisture presence during application.[30,36] However, studies show that glass ionomer fissure sealants have lower retention and higher microleakage rates than resin based fissure sealants.[31-34]

D) RESIN MODIFIED GLASS IONOMER FISSURE SEALANT

Resin modified glass ionomer materials contain modified polyacrylic acid polymers which can copolymerize with other resins in addition to their contribution in the conventional setting reaction.[64] Studies showed that resin modified glass ionomer fissure sealants have lower retention than the resin based fissure sealant.[40] But in comparison with glass ionomer fissure sealant, resin modified glass ionomer fissure sealants have better retention than conventional glass ionomer fissure sealant.[42]

RESEARCH DESIGN AND METHODS

A) SAMPLE SIZE CALCULATION:

A power calculation was conducted using the statistical software package R (Version 2.11.1). Based on the values of Bahrololoomi et al.[65] for the dry conditions, and the values of Bassir et al.[66] for the difference between the dry and contaminated conditions, a sample size of n=12 per group was adequate to obtain a Type I error rate of α =5% and a power greater than 80%.

B) EXPERIMENTAL DESIGN:

This study was designed to be a blinded randomized controlled in-vitro experiment. Extracted human third molars with deep pits and fissures, free from caries and restoration were included in this study. All fissures were cleaned with fluoride-free pumice using a prophylaxis brush at low speed. The teeth were randomly distributed into four groups using the statistical software package R (version 2.13.1) (Table 1). Sealants were placed according to the manufacturer's instructions as follows:

Group 1: Ultra-Etch® 35% (Ultradent Products, Inc. South Jordan, UT) was applied to fissures for 20 seconds. Then the tooth was thoroughly rinsed with water and air dried. UltraSeal XT hydro sealant material was applied and cured with a light-emitting diode (LED) curing light for 20 seconds.

Group 2: Ultra-Etch® 35% (Ultradent Products, Inc. South Jordan, UT) was applied to fissures for 20 seconds. The tooth was thoroughly rinsed with water and air dried. Then the tooth was completely immersed in artificial saliva {containing mmoles/L:$CaCl_2$ (0.7), $MgCl_2 \cdot 6H_2O$ (0.2), KH_2PO_4 (4.0), KCl (30), NaN_3 (0.3), and HEPES buffer (20), protease inhibitors (mmoles/L): benzamidine HCl (2.5), εamino-n-caproic acid (50), Nethylmaleimide (0.5), and phenylmethylsufonyl fluoride (0.3)}[67] for 5 seconds and air dried for 5 seconds. UltraSeal XT hydro sealant material was then applied and cured with LED curing light for 20 seconds.

Group 3: Self-etching primer (Shofu, Inc. San Marcos, CA) was applied for 5 seconds to clean the tooth. The tooth was air dried for 5 seconds. BeautiSealant was applied and cured with LED curing light for 10 seconds.

Group 4: Self-etching primer (Shofu, Inc. San Marcos, CA) was applied for 5 seconds to clean the tooth. The tooth was air dried for 5 seconds. Then the tooth was completely immersed in artificial saliva {containing mmoles/L: $CaCl_2$ (0.7), $MgCl_2 \cdot 6H_2O$ (0.2), KH_2PO_4 (4.0), KCl (30), NaN_3 (0.3), and HEPES buffer (20) with protease inhibitors (mmoles/L): benzamidine HCl (2.5), εamino-n-caproic acid (50), Nethylmaleimide (0.5), and phenylmethylsufonyl fluoride (0.3)}[67] for 5 seconds and air dried for 5 seconds. BeautiSealant was then applied and cured with LED curing light for 10 seconds.

C) THERMOCYCLING:

All samples were subjected to a thermocycling regimen of 10000 cycles between 5°C and 55°C water baths with a 15-second dwell time using a thermal cycler (Proto-tech. Portland, OR) (Figure 1). The tooth apex was covered with sticky wax. The whole tooth was covered with two layers of nail varnish leaving a 1mm window around the sealant. After the thermocycling procedure, all samples were immersed in 1-percent methylene blue dye for 24 hours at 37°C and in distilled water for 24 hours at 37°C.

The specimen was embedded into orthodontic resin (Dentsply Caulk. Milford, RE) (Figure 2) and each specimen was sectioned in a buccolingual direction into three sections (mesial, central, and distal sections) using isomet diamond wafering blades in a low speed sectioning wheel isomet 1000 (Buehler, Germany) (Figure 3).

The depth of dye penetration was evaluated using a stereomicroscope (Olympus SZX16. Tokyo, Japan) by a blind investigator who was unaware of the type of the sealant material, and was recorded according to the dye penetration score system (Figures 4-7) described as follows:

Score 0=no dye penetration.

Score 1=dye penetration restricted to the outer half of the sealant-fissure interface.

Score 2=dye penetration to the inner half of the sealant-fissure interface.

Score 3=dye penetration into the underlying fissure.

D) STATISTICAL ANALYSIS:

Counts and proportions were reported. All data were analyzed with the Kruskal-Wallis and Mann-Whitney U tests. A p-value of ≤ 0.05 was considered statistically significant for the Kruskal-Wallis test. The Bonferroni correction for the Mann-Whitney U tests was performed and a p-value of ≤ 0.0125 was considered statistically significant. Statistical analysis was performed using SPSS Version 21 (IBM Corporation. Armonk, NY).

RESULTS

All 48 teeth were examined for microleakage. Table 2 illustrates the distribution of microleakage scores amongst the groups, taking the maximum microleakage score for each tooth. UltraSeal XT Hydro in dry condition (Group 1) had the best result with no dye penetration (score 0) in all the teeth (100%). Group 2 (UltraSeal XT Hydro in saliva contaminated condition) had no microleakage (score 0) in 50% of the teeth, with no scores of 2 or 3 in the group. For BeautiSealant in dry condition (Group 3), 16.67% had a score of 0; 58.33% had a score of 1; 8.33% had a score of 2; 16.67% had a score of 3 (where the dye reaches the underlying fissure at the base of the sealant). The highest microleakage scores were in Group 4 (BeautiSealant in saliva contaminated condition) with 58.33% of the teeth getting score 3, and only one out of twelve teeth having no microleakage (score of 0). Table 3 shows the median microleakage and interquartile range for each section in the groups.

The Kruskal-Wallis test revealed statistically significant differences in microleakage ($p<0.001$) among the four groups using the maximum microleakage score for each tooth. Also, the Kruskal-Wallis test found statistically significant differences when comparing the mesial, central and distal sections for the groups ($p=0.001$, $p=0.001$ and $p<0.001$, respectively) (Table 4).

The Mann-Whitney U test found statistically significant differences between Groups 1 and 3 ($p<0.001$) and between Group 2 and 4 ($p=0.001$) when using the maximum microleakage score for each tooth (Table 5). There was no statistically significant difference between Groups 1 and 2 or between Groups 3 and 4. Table 6 shows the results of the Mann-Whitney

U tests among the four groups in all three sections. There was a statistically significant difference between Groups 1 and 3 in the mesial and distal sections (p=0.005).

DISCUSSION

Despite all the preventive techniques used to decrease the incidence of dental caries, it remains the most common childhood disease, with 90% of dental caries found to be pit and fissure caries.[1,2,7,9] The morphology of the occlusal surfaces allows for plaque stagnation. Moreover, the anatomy of the pits and fissures that reaches the dentinoenamel junction makes the occlusal surfaces the most caries susceptible surface in the tooth.[12-15]

Researchers found that pits and fissures sealant is the most effective method in preventing occlusal caries.[19,21,22] The caries preventive effect of pit and fissure sealant materials depends mainly on the sealing ability of the materials to prevent microleakage into the underlying fissures.[25] Measurements of microleakage have been used in the literature to evaluate the marginal sealing ability of sealant materials.[1,50-54] The most commonly used fissure sealant is the hydrophobic resin based fissure sealant.[24] The hydrophobic nature of resin based fissure sealant makes saliva contamination the most common risk factor in sealant failure.[26,27]

The purpose of this study was to compare two types of sealant materials (UltraSeal XT hydro and BeautiSealant) for microleakage and to evaluate the effects of saliva contamination on the microleakage of both types of sealants. UltraSeal XT hydro is a hydrophilic resin based sealant, which the company claims will eliminate the need for use of a drying agent before application and ensures marginal retention. BeautiSealant is a resin based sealant that contains Shofu's proprietary Surface Pre-Reacted Glass (S-PRG filler particles). The unique structure form of BeautiSealant will supposedly protect the glass core from the damaging effects of moisture and improve long term durability.

Our results showed that UltraSeal XT hydro had the best results when it was placed in dry conditions. However, there were no statistically significant differences between UltraSeal XT hydro in dry condition (Group 1) and UltraSeal XT hydro in saliva contaminated condition (Group 2). Furthermore, there were no statistically significant differences between BeautiSealant in dry condition and BeautiSealant in saliva contaminated condition. The results showed that BeautiSealant had higher microleakage scores than UltraSeal XT hydro in both dry and saliva contaminated conditions. The worst microleakage scores were found to be in Group 4 (BeautiSealant in saliva contaminated condition).

The differences in microleakage scores between UltraSeal XT hydro groups and BeautiSealant groups may in part due to the methods of enamel preparation. While UltraSeal XT hydro uses 35% phosphoric acid solution for enamel preparation, BeautiSealant uses a self-etching primer instead of the phosphoric acid. Our results are in agreement with the findings of Parco et al.[68]; they found that the self-etching sealant, in both dry and contaminated condition, had more microleakage than the traditional phosphoric acid-etched sealant. Similar results were found by Tehrani et al[69]; they found that the worst microleakage scores occurred when self-etching primer was used in comparison with the microleakage scores when the traditional phosphoric acid-etch was used. Our results showed that the use of self-etching primer might be insufficient for enamel preparation before sealant placement, which is similar to the findings of two previous studies.[70,71]

The limitations of this study are:

- In-vitro studies are unable to fully simulate actual conditions of the oral cavity.
- The differences in pits and fissure morphology between first and third molars.

- The differences in viscosity and components between artificial saliva and natural human saliva.
- The differences in time intervals between the tooth extraction and start of the experiment.

CLINICAL IMPLICATION:

The measurement of microleakage has been used in previous studies to assess the marginal sealing ability of sealant materials. Our results could help dentists to choose between the two brands that we tested in our study.

In some clinical situations, moisture control may be difficult to achieve when placing sealants, such as placing sealants on young uncooperative children, partially erupted molars, or in school based sealant programs. In these situations, the provider may decide to delay the placement of the sealant, or may not place them at all. There is a need to find a hydrophilic resin based sealant materials that could work in these situations. There was not statistically significant evidence of a difference in microleakage between UltraSeal XT hydro in dry and contaminated conditions.

FUTURE STUDIES:

Future studies should involve long term randomized controlled clinical trials to check retention rates of the new hydrophilic sealants in the oral environment.

CONCLUSIONS

With the consideration to the limitations of an in-vitro study, we can conclude that the hydrophilic resin based sealant (UltraSeal XT hydro) had better microleakage scores than sealants containing surface pre-reacted glass particles (BeautiSealant) in both dry and saliva contaminated conditions. There was not statistically significant evidence of a difference in microleakage between UltraSeal XT hydro in dry and contaminated conditions.

APPENDIX A

Table 1: Groups Characteristics

Product	***Non-contaminated condition***	***Saliva contaminated condition***
UltraSeal XT hydro	Group 1	Group 2
BeautiSealant	Group 3	Group 4

Table 2: Dye penetration scores for all the groups taking the maximum score for each tooth*

	Dye penetration scores			
Group	**0**	**1**	**2**	**3**
1	12 (100%)	0 (0%)	0 (0%)	0 (0%)
2	6 (50%)	6 (50%)	0 (0%)	0 (0%)
3	2 (16.67%)	7 (58.33%)	1 (8.33%)	2 (16.67%)
4	1 (8.33%)	3 (25%)	1 (8.33%)	7 (58.33%)

* Percentages might not sum to 100% due to rounding.

*Table 2: Distribution of microleakage scores (score 0= no dye penetration, score 1= dye penetration restricted to the outer half of the sealant-fissure interface, score 2=dye penetration to the inner half of the sealant-fissure interface, score 3=dye penetration into the underlying fissure at the base of the sealant) among four experimental groups (Group 1= Ultra Seal XT Hydro in dry condition, Group 2= Ultra Seal XT Hydro in saliva contaminated condition, Group 3= BeautiSealant in dry condition, Group 4= BeautiSealant in saliva contaminated condition)

Table 3: Median and interquartile range of microleakage for mesial, central and distal sections in the four groups

Groups	Mesial section Median ± IQR	Central section Median ± IQR	Distal section Median ± IQR
1	0 ± 0	0 ± 0	0 ± 0
2	0 ± 0	0 ± 1	0 ± 0
3	1 ± 1.5	0 ± 1	1 ± 1
4	1 ± 3	1 ± 2	1 ± 3

Table 4: Results of Kruskal-Wallis tests by section

	Mesial	Central	Distal
P- value*	0.001	0.001	<0.001

* A p-value ≤.05 was considered statistically significant

Table 5: Results of Mann-Whitney U tests using the maximum microleakage score

Groups	Groups	P-value*
1	2	0.039
3	4	0.068
1	3	**<0.001**
2	4	**0.001**

*A p-value ≤.0125 was considered statistically significant

Table 6: Results of Mann-Whitney U tests in mesial, central and distal sections

Groups	Groups	P-value*		
		Mesial	Central	Distal
1	2	0.514	0.089	0.514
3	4	0.630	0.052	0.514
1	3	**0.005**	0.089	**0.005**
2	4	0.045	0.033	0.020

*A p-value ≤.0125 was considered statistically significant

APPENDIX B

Figure 1: Thermal cycler (Proto-tech. Portland, OR)

Figure 2: Samples embedded into orthodontic resin (Dentsply Caulk)

Figure 3: Low speed sectioning wheel isomet 1000 (Buehler, Germany)

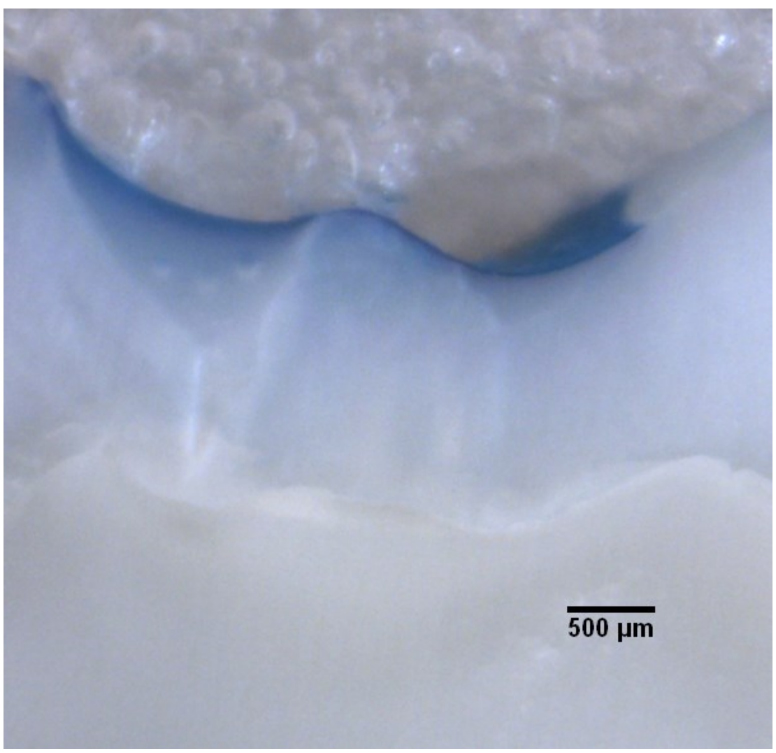

Figure 4: Score 0=no dye penetration.

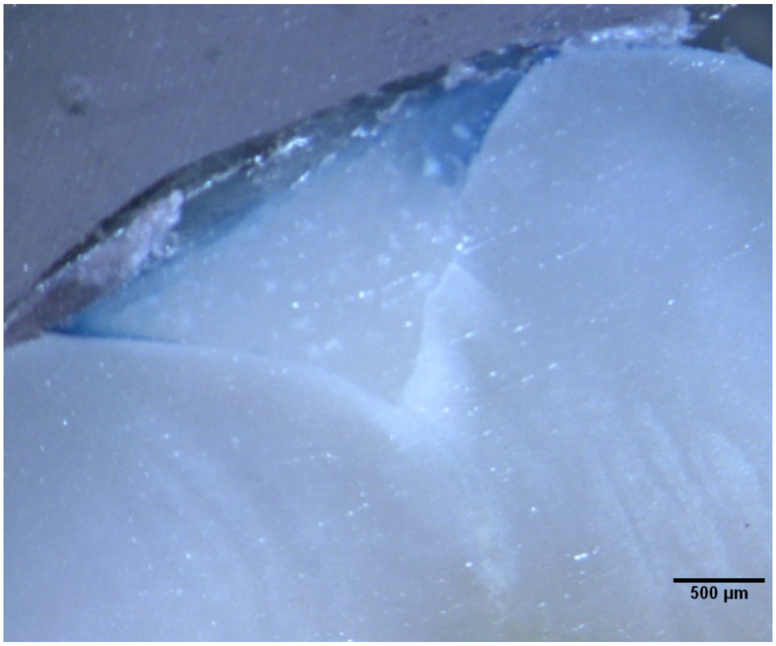

Figure 5: Score 1=dye penetration restricted to the outer half of the sealant-fissure interface.

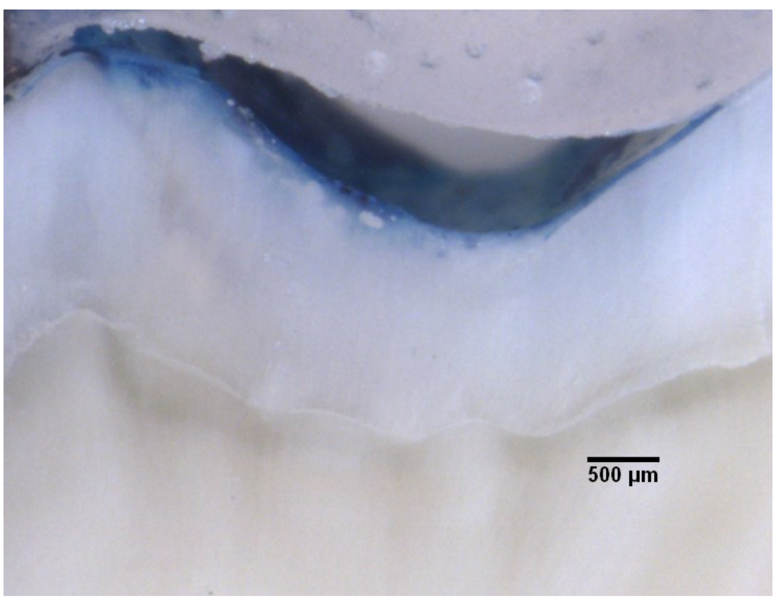

Figure 6: Score 2=dye penetration to the inner half of the sealant-fissure interface.

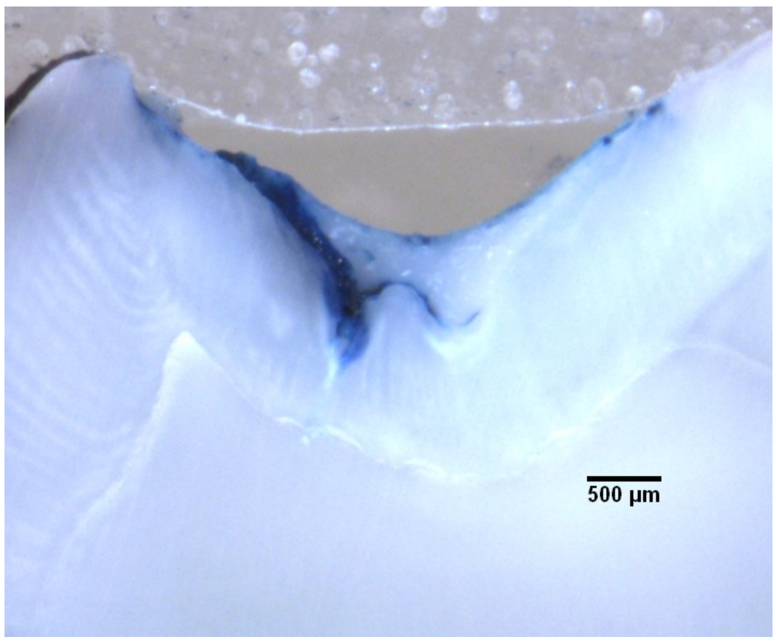

Figure 7: Score 3=dye penetration into the underlying fissure.

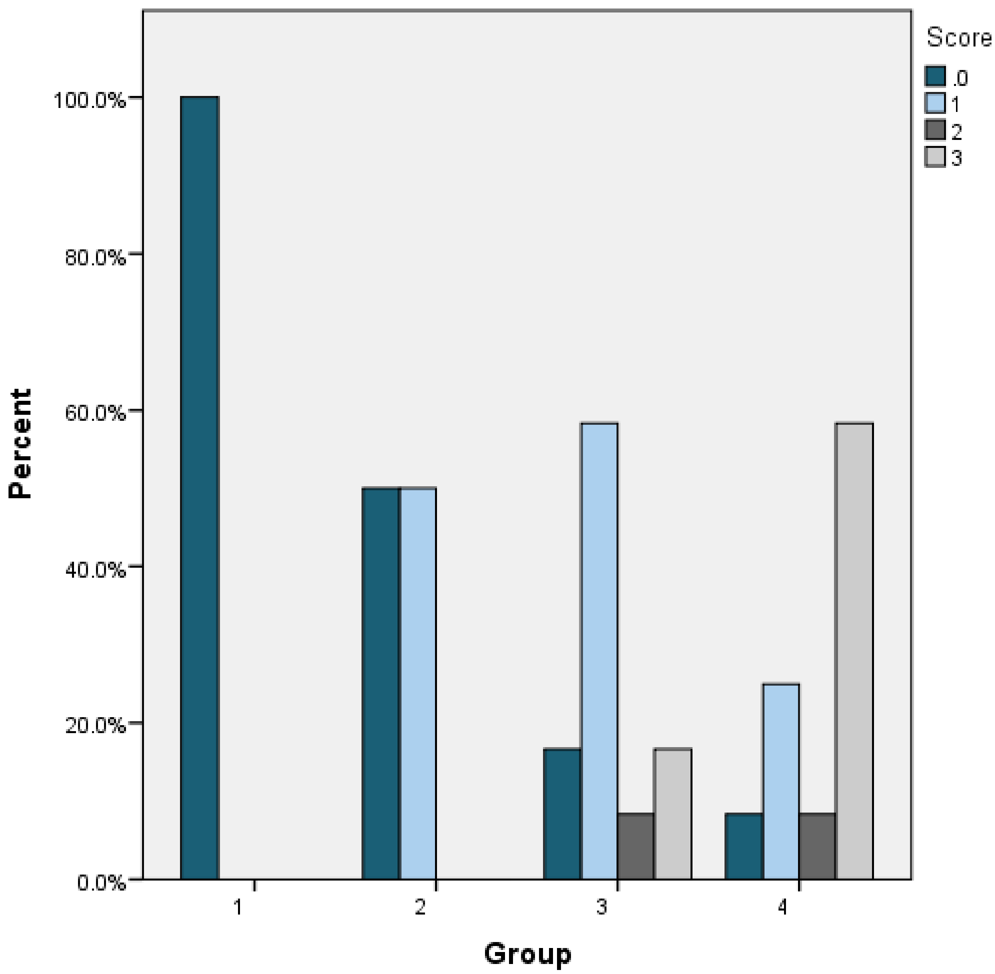

Figure 8: Distribution of microleakage scores [(score 0= no dye penetration, score 1= dye penetration restricted to the outer half of the sealant-fissure interface, score 2=dye penetration to the inner half of the sealant-fissure interface, score 3=dye penetration into the underlying fissure)] among different experimental groups [(group 1= Ultra Seal XT Hydro in dry condition, group 2= Ultra Seal XT Hydro in saliva contaminated condition, group 3= BeautiSealant in dry condition, group 4= BeautiSealant in saliva contaminated condition)]

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