Effect of Light Cure Methods for Intermediate Adhesive Layer on Microleakage of Sealants. An *in Vitro* Study

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Background: *The objective of this study was to determine the effect of an adhesive layer and its photopolymerization on the microleakage of sealants.* ***Study design:*** *0.5mm-deep standardized fissurectomies were performed on extracted molars (n = 72). Teeth were randomly assigned to 3 different sealant materials (n = 24/group). Teeth were further divided to receive sealants with or without an adhesive layer. Each sealant with adhesive was also divided into two groups: adhesive was light-cured separately or light cured together with the sealant. Following thermocycling, microleakage was assessed using dye penetration and image analysis. Data was analyzed using ANOVA and Tukey's studentized ranged HSD tests.* ***Results:*** *Microleakage was not affected by type of sealant material (p>0.05) but was significantly influenced by application (p<0.05). Overall, placement of sealants without adhesive displayed greater microleakage than sealants with uncured adhesive (p<0.05). Within individual sealant types, this difference was only significant for Ultraseal XT (p<0.05). Sealants bonded with and without prior light curing did not show a significant difference in levels of leakage (Tukey's Studentized Range Test, p>0.05).* ***Conclusion:*** *An adhesive layer should be placed beneath sealants, but whether it should be light cured or left uncured before sealant placement varies by the sealant type.*

Keywords: *light curing, dental adhesives, polymerization, image analysis, pit and fissure sealants.*

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INTRODUCTION

The American Dental Association (**ADA**) advocates that pit and sealant fissures are effective and safe to prevent or arrest carious lesions.[1] Resin-based sealants are significantly more effective in preventing caries in first permanent molars compared to no sealant[2] and fluoride varnish.[3] Based on evidence from at least one randomized controlled trial[4], the ADA recommends that sealant retention can be improved with an intermediate adhesive layer between an acid-etched surface and the sealant material.[5] Initially, bonding agents were recommended to offset the humidity of the oral environment.[6] However, others noted that an improvement in retention even in a dry environment may be due to increased resin monomer infiltration into nano-retentive areas of etched enamel as a result of the shorter chain length.[7]

Despite this recommendation for bonding agent use prior to sealant placement, evidence regarding efficacy has been mixed. Clinical trials utilizing bonding agents have found positive effects,[4,8-12] while others have found no significant effect when retention and caries prevention are primary outcomes.[13-17] Despite this conflicting data, a recent systematic review and meta-analysis concluded that the use of adhesive systems beneath fissure sealants can increase the retention.[18]

In vitro studies show similar conflicting conclusions. Hitt *et al* [6] found significantly greater shear bond strength when a 5th generation adhesive system was used in normal and contaminated conditions, while Choi *et al* [19] reported significantly improved shear strength when primer was used only under moist conditions. Likewise, some in vitro studies have shown reduced microleakage when bonding agents were used under sealants [7,20-22], while others have shown no effect.[23,24]

Despite the abundance of studies performed on the use of bonding agent, there has been little discussion regarding recommendations to cure the bonding agent separately or to cure it with the sealant. Roughly half of *in vivo* studies have cured the bonding agent separately[9,11,13,15,16] where the remaining have cured it in a single step with the sealant.[4, 8],[14,17,25,] *In vitro* studies have predominately cured bonding agent[6,7,21,23,24] with some exceptions[20], and not all studies have specified the curing status of the bonding agent.[10, 22] A recent study found that the addition of bonding agent without a separate curing step reduced microtensile bond strength.[26] However, the impact of light curing bonding agents on microleakage is unknown.

The objective of this *in vitro* study was to investigate the microleakage of three commercial sealants, when (a) sealants were used with and without bonding agents, and (b) the bonding agent was light-cured or left uncured before placement of sealant. The two-fold null hypothesis is that the addition of bonding agent will not affect microleakage, and that the difference in the application mode of the bonding agent (cured vs. uncured) will not affect sealing properties of the tested sealant materials.

MATERIALS AND METHOD

Specimen preparation

This study was exempted from oversight by the Institutional Review Board. Sample size calculation was performed based on previous data.[27] Sound, caries-free extracted permanent molars (n=72) were randomly selected from a bank of teeth stored in 10% sodium hypochlorite solution at room temperature. After surface debridement with hand scaling instruments and dry brushing, the teeth were examined under 10X magnification to discard those with any visible structural defects, cracks, or incipient lesions. The specimens were randomly assigned to 3 groups (n=24/group) with regard to the sealant material tested: group 1. Clinpro Sealant (3M/ESPE, St. Paul, MN); group 2. Ultraseal XT Hydro (Ultradent, South Jordan, UT); and group 3. Fluorshield VLC (DENTSPLY, York, PA). The composition of test materials is shown in Figure 1. Each group was further divided into 3 subgroups (n = 8/group) based on the sealant application protocols used: (a) light-cured bonding agent; (b) "uncured" bonding agent; and (c) no bonding agent control with sealant only.

A 0.5mm-deep fissure was prepared using high-speed, water cooled diamond bur throughout the central grooves of all specimens in effort to make the fissures of all specimens analogous. All fissures were acid-etched using 35% phosphoric acid gel (UltraEtch, Ultradent, South Jordan, UT) for 30 seconds, rinsed for 15 seconds, and air-dried for 5 seconds. The specimens within the light-cured and "uncured" groups received a layer of Adper ™ Single Bond Plus (3M/ESPE, St. Paul, MN, USA) applied throughout the grooves of the specimens with a microbrush for 20 seconds and thinned with compressed air for 5 seconds. For each sealant material, the bonding agent in the light-cured subgroup was photopolymerized with a LED curing unit (Elipar Deep Cure, 3M/ESPE, St. Paul, MN, USA) separately for 20 seconds prior to applying sealant material. For the "uncured" subgroups, the bonding agent was not light cured in a separate step but was cured together with sealant materials. In all groups, the tip of the light source was placed on the occlusal cusps to minimize the distance from the occlusal surface. All sealant materials were light-cured for 40 seconds. An explorer was used to ensure that the margins were smooth and the sealants were free of voids.

Figure 1. Composition of the tested materials.

Material (Manufacturer)	Composition
Clinpro Sealant (3M ESPE, St. Paul, MN)	TEGDMA, BISGMA, Silane treated silica Tetrabutylammonium Tetrafluoroborate, Diphenylidonium, Hexafluorophospate, Ethyl 4-dimethyl aminobenzoate, Titanium dioxide, Hydroquinone
Ultraseal XT hydro Sealant (Ultradent, South Jordan, UT)	TEGDMA, UDMA, Aluminum Oxide, Methacrylic Acid, Titanium Dioxide, Sodium Monofluorophosphate
Fluorshield VLC Sealant (DENTSPLY,York, PA)	50% by weight inorganic filler composed of Urethane Bis-GMA, Dimethacrylate resins, Barium aluminoborosilicate glass, sodium fluoride, photoinitiator, photoaccelerators, silicon dioxide
Adper Single Bond Plus (3M ESPE, St. Paul, MN)	Ethanol, BISGMA, Silane Treated Silica, HEMA, Glucerol 1,3-Dimethacrylate, Copolymer of acrylic and itaconic acids, water, UDMA, EDMAB
UltraEtch (Ultradent, South Jordan, UT)	35% phosphoric acid

TEGDMA: Triethylene Glycol Dimethacrylate; BISGMA: bisphenol-A-diglycidyl ether dimethacrylate; HEMA: 2-hydroxyethyl methacrylate; UDMA: urethane dimethacrylate; EDMAB: Ethyl 4-Dimethylaminobenzoate.

Microleakage Test

All specimens were thermocycled between 5-55°C for 2,000 cycles. Microleakage was assessed with a dye penetration method.[27] Accordingly, the unsealed surfaces of the specimens were coated with acrylic nail polish with a 1mm window left around the sealant, stained in 2% basic fuchsin for 24 hours, rinsed, and air dried. The samples were sectioned buccolingually into 1mm slabs, and photographed at 4x magnification with a digital camera (SPOT Insight, Sterling Heights, MI) mounted on a polarizing light microscope (i50, Nikon Corp., Japan). The extent of microleakage degrees was scored on a value scale of 0 to 4[27] where:

0=no penetration;

1=¼ penetration;

2= ½ penetration;

3= ¾ penetration; and

4=penetration to the bottom of the fissure for both the buccal and distal portion of the sample.

The microleakage depths were also measured and calculated digitally using ImageJ image analysis software (ImageJ, U. S. National Institutes of Health, Bethesda, Maryland) as follows:

Image Analysis Microleakage = Penetration Depth/Sealant Depth ×4

Statistical Analysis

For both the score-based and the digital measurements, microleakage values were analyzed using ANOVA and Tukey's studentized ranged (HSD) test. Pearson correlation coefficients test and Kappa statistics were used to analyze the agreement between the score-based and the digital measurements. All statistical analyses were made with SAS software (V 9.4, SAS Inc., Cary, NC).

RESULTS

The examples of microleakage measured by visual scoring and image analysis are shown in Figure 2. The microleakage values of the test groups and subgroups are presented in Table 1 as Mean ± standard deviation. The microleakage values measured by visual scoring and image analysis were highly correlated (Pearson correlation coefficient = 0.99037). Likewise, Kappa statistics indicated good agreement (0.8105) between both methods.

The type of sealant material had no significantly different effect on microleakage (ANOVA, $p>0.05$). However, the application mode significantly influenced the overall sealing effectiveness with uncured bonded sealants displaying significantly less microleakage compared to conventional sealants (ANOVA, $p<0.05$). There was no significant difference between microleakage in cured and uncured bonded sealants or between cured bonded sealants and conventional sealants. For each sealant material tested, the (conventional)

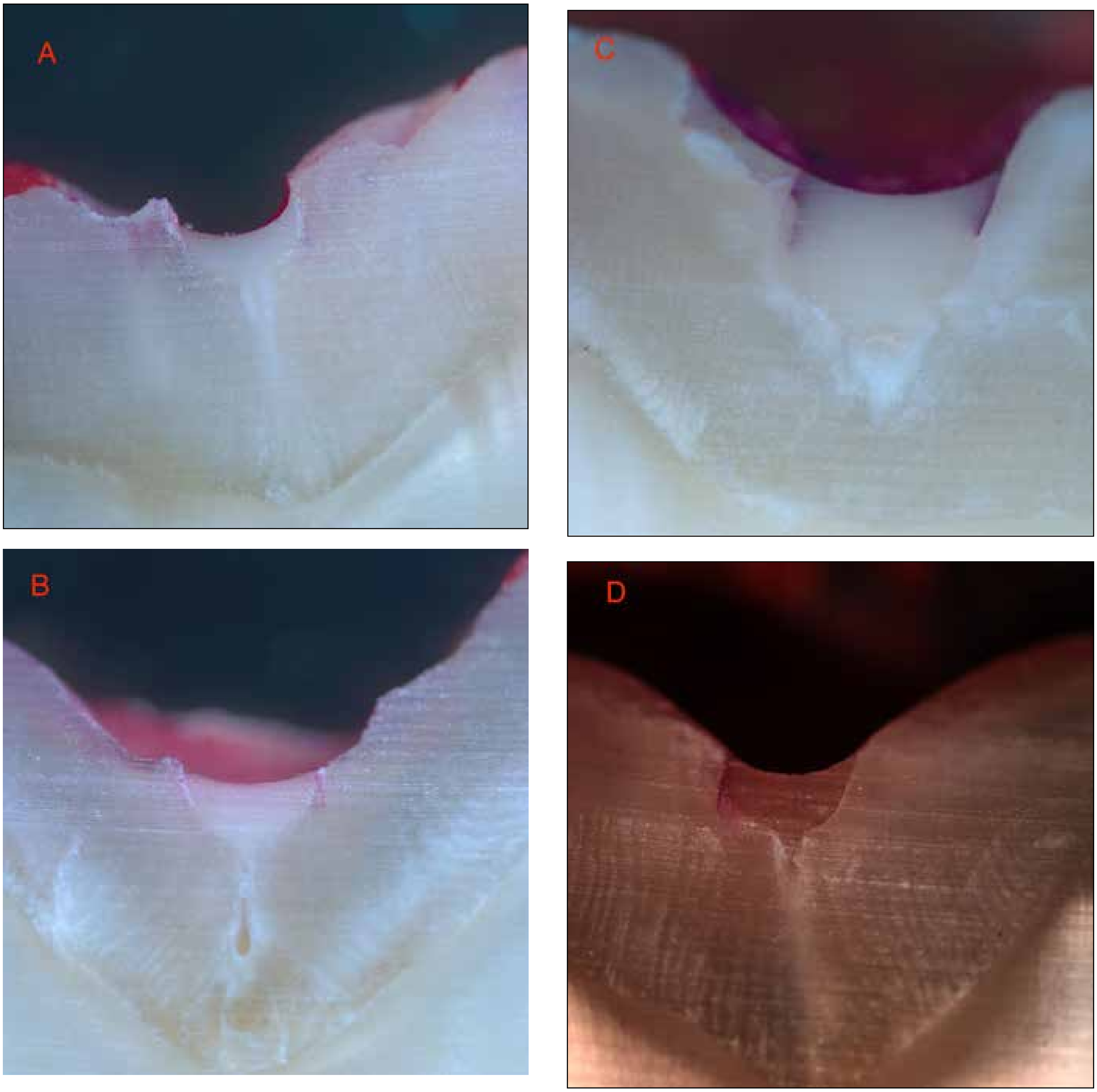
Table 1. Mean microleakage, standard deviation, and confidence intervals using both measurement methods. Image analysis was performed using software measurements and the formula "Image Analysis Microleakage = Penetration Depth/Sealant Depth ×4". Visual scoring was assigned through assessment of the images without software utilization.

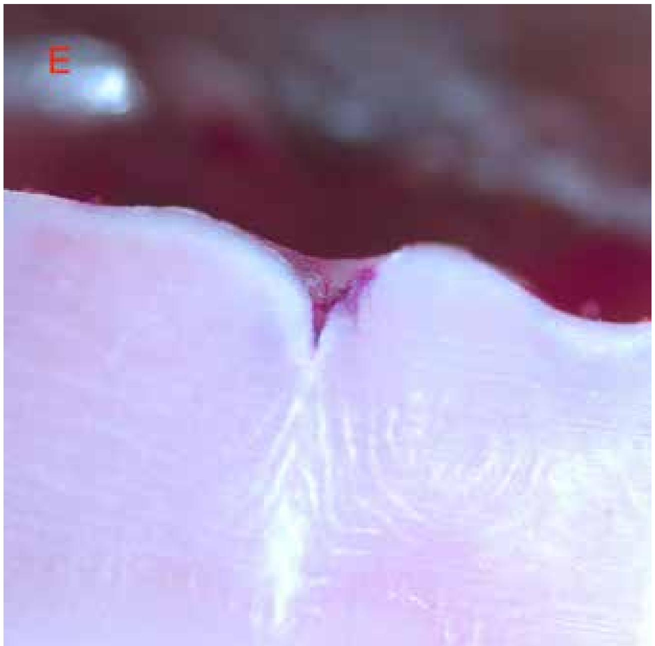
Sealant	Adhesive Application*	N	Measurement Type	Mean	Std. Dev.	Lower 95% CL for Mean	Upper 95% CL for Mean
Clinpro	Cured	8	Image Analysis	0.97	0.57	0.49	1.44
			Visual Scoring	0.98	0.53	0.54	1.43
	Uncured	8	Image Analysis	0.62	0.47	0.22	1.07
			Visual Scoring	0.68	0.53	0.24	1.13
	No adhesive (Control)	8	Image Analysis	1.04	0.65	0.50	1.59
			Visual Scoring	1.07	0.63	0.54	1.60
Fluorshield	Cured	8	Image Analysis	0.37	0.47	-0.02	0.76
			Visual Scoring	0.37	0.49	-0.04	0.79
	Uncured	8	Image Analysis	0.38	0.4	0.05	0.71
			Visual Scoring	0.42	0.41	0.08	0.76
	No adhesive (Control)	8	Image Analysis	0.92	0.79	0.26	1.58
			Visual Scoring	0.96	0.80	0.29	1.63
Ultraseal XT	Cured	8	Image Analysis	0.74	0.73	0.13	1.34
			Visual Scoring	0.76	0.71	0.17	1.35
	Uncured	8	Image Analysis	0.17	0.2	-0.00042	0.33
			Visual Scoring	0.16	0.21	-0.01	0.34
	No adhesive (Control)	8	Image Analysis	1.02	0.55	0.57	1.48
			Visual Scoring	1.05	0.56	0.58	1.52

*Cured= The adhesive is light-cured in separate step.

Uncured = The adhesive and the sealant are light cured together in a single step.

Figure 2. Examples of microleakages by visual scoring and measured by image analysis. (A) No leakage, Clinpro sealant only (B) score 1, Clinpro sealant only, (C) score 2, Clinpro cured bonding agent:, (D) score 3, Fluoroshield sealant only: (E) score 4, Clinpro uncured bonding agent





placement of sealant without bonding agent displayed greater leakage than the bonded sealants, but this difference was only significant in the Ultraseal XT group (Tukey's Studentized Range Test, $p<0.05$). For each sealant material, placement of an adhesive layer with and without prior light curing did not show difference in levels of leakage (Tukey's Studentized Range Test, $p>0.05$).

DISCUSSION

Microleakage is defined as the clinically undetectable passage of bacteria and fluids between a cavity wall and a restorative material.[28] The long-term success of a sealant depends on both its clinical retention and its ability to resist microleakage.[21] Sealant retention is strongly influenced by microleakage, since leakage around the sealant will cause loss of retention over the time. It is also possible that a sealant may exhibit good bond strength but has microleakage, which would eventually lead to secondary caries long before the sealant is lost.

Traditionally microleakage has been determined by a conventional scoring system for grading the extent of dye penetration. Previous studies have utilized a more objective approach utilizing image analysis.[21,29] This study compared the outcomes of the conventional scoring system with image analysis, and the results confirmed that both methods were highly correlated. Nevertheless, the authors believe that the image analysis method is more objective and should be recommended as a new standard for determination of microleakage.

In this study, curing bonding agent prior to sealant placement did not yield better sealing effectiveness compared with uncured bonded sealants. These results necessitate acceptance of the null hypothesis in part. Thus, it seems that the effect of light-curing methods (light-curing the bonding agent and sealant separately or curing them together) on sealing effectiveness of pit and fissure sealants depends strongly on the type of materials used. Accordingly, the chemical composition and physical properties (e.g., viscosity, refractive index) of both the adhesive and the sealant will likely influence the light cure effect. Provided that curing the bonding agent together with the sealant has no effect on the clinical performance of bonded fissure sealants, the latter approach may help save time and simplify the process by omitting another step. Literature on sealant outcomes remains divided on curing the bonding agent [6,7,9,11,13-16,21,23,24] versus curing with sealant [4, 8,17,19,20,25,,] with no clear difference in outcomes. The present study confirms that this effect is indeed material dependent. In the present study uncured bonding agent yielded significantly less leakage than control when Ultraseal XT was used. This may be explained by mixing of the unfilled bonding agent with the more highly-filled sealant thus increasing the wetting properties of the sealant. When using highly-filled sealant materials, co-curing as a single entity resulted in ultimately better sealing properties.

Here, the sealants placed with an intermediate layer of bonding agent had reduced microleakage compared to conventional "unbonded" sealants, but this effect was only significant in the Ultraseal XT group. Thus, the null hypothesis should be rejected in part only for the latter group. The improvement of sealing properties in bonded sealants is in agreement with Hebling *et al* [20], who reported significantly reduced leakage values when a bonding agent was used in the presence of saliva contamination. Likewise, Feigal *et al* [4] and Pinar *et al* [17] observed a reduction in marginal discoloration when a bonding agent was used, probably as a result of reduced microleakage. Such reduction may also be the reason that Feigal[4] and McCafferty[8] found improved retention when bonding agents were used. This study confirms that the inclusion of an intermediary layer of bonding agent prior to sealant application is a beneficial approach.

The primary limitation of this study was that it was conducted in a laboratory setting with an optimal environment with no moisture contamination. The impact of the addition of bonding agent is likely more evident if the sealant is being placed in an in vivo setting compared to the in vitro setting of the present study. Feigal *et al* [4], found that in a moist environment, bonding agent improved retention of the sealants in the buccal and lingual grooves, where early loss of sealants is most likely to occur .[30] Other clinical studies have shown similar improvement in retention effects.[7,19, 20] It is notable that previous authors have hypothesized that the addition of bonding agent is only advantageous in a humid environment to offset the moisture contamination, but this study has also shown a benefit even in ideal laboratory settings.[6] It is important to note that this study did not use materials based on the manufacturer's recommendations in order to standardize procedures. The manufacturer's recommendation for Fluorshield VL is a 60 second etch versus a 20 second etch for the other two materials. However, using this increased etching time may have resulted in reduced microleakage for this material skewing the results. This may lead to results different than typical clinical practice. Finally, permanent teeth that are newly erupted and have sealants placed contain prismless enamel that does not wear away for months or years which may affect the bonding. The teeth used in this study were a mix of erupted and unerupted permanent molars so this impact could not be fully studied. As a result, it is possible that the variability in this sample could have skewed the data, although an attempt for standardization was made by fissurotomies.

While it is evident that the use of bonding agent improves efficacy of sealants, dentists must rank the importance of microtensile strength and microleakage. As shown previously , when bonding agent was cured independently of the sealant material, microtensile strength properties were superior to specimens with the bonding agent and sealant material cured simultaneously.[26] The methods of this study differed from the McMurphy study in that different materials, including bonding agents, were used and manufacturer's instructions were followed.[26] Ultimately, dentists must use their clinical judgement to decide whether they believe a well retained sealant with increased possibility of microleakage is better for a patient than a sealant with superior sealing properties with less optimal retentive properties.

CONCLUSIONS

Within the experimental limitations of an in vitro study, the following conclusions were drawn:

1. Overall, the addition of uncured bonding agent results in significantly less microleakage compared to sealant placement without bonding agent.
2. The effect of light cure methods on microleakage (curing the bonding agent and sealant separately or curing them together) varies by the sealant materials used.
3. Measurement of microleakage by subjective scoring and image analysis were highly correlated.

REFERENCES

Wright JT, Tampi MP, Graham L, Estrich C, Crall JJ, Fontana M, Gillette EJ, Nový BB, Dhar V, Donly K, Hewlett ER, Quinonez RB, Chaffin J, Crespin M, Iafolla T, Siegal MD, Carrasco-Labra A. Sealants for Preventing and Arresting Pit-and-fissure Occlusal Caries in Primary and Permanent Molars. J Am Dent Assoc;147(8):631-45. 2016.

2. Ahovuo-Saloranta A1, Forss H, Hiiri A, Nordblad A, Mäkelä M. Pit and fissure sealants versus fluoride varnishes for preventing dental decay in the permanent teeth of children and adolescents. Cochrane Database Syst Rev.; 18(1):CD003067. 2016
3. Ahovuo-Saloranta A, Forss H, Walsh T, Hiiri A, Nordblad A, Makela M, Worthington HV. Sealants for preventing dental decay in the permanent teeth. Cochrane Database Syst Rev.; 28(3): CD001830. 2013.
4. Feigal RJ, Musherure P, Gillespie B, Levy-Polack M, Quelhas I, Hebling J. Improved sealant retention with bonding agents: a clinical study of two-bottle and single-bottle systems. J Dent Res;79(11):1850-1856. 2000.
5. Beauchamp J, Caufield PW, Crall JJ, et al. Evidence-based clinical recommendations for the use of pit-and-fissure sealants: a report of the American Dental Association Council on Scientific Affairs. J Am Dent Assoc ;139(3):257-268. 2008.
6. Hitt JC, Feigal RJ. Use of a bonding agent to reduce sealant sensitivity to moisture contamination: an in vitro study. Pediatr Dent;14(21):41-6. 1992.
7. Meller C, Reichenmiller K, Schwahn C, Samietz S, Blunck U. Resin-based Pit-and-Fissure Sealants: Microleakage Reduction and Infiltration Enhancement Using a Bonding Agent. J Adhes Dent;17(1): 59–65. 2015.
8. McCafferty J, O'Connell AC. A randomised clinical trial on the use of intermediate bonding on the retention of fissure sealants in children. Int J Paediatr Dent;26(2):110-5. 2016.
9. Nogourani MK, Janghorbani M, Khadem P, Jadidi Z, Jalali SA. 12-month clinical evaluation of pit-and-firssure sealants placed with and without etch-and-rinse and self-etch adhesive systems in newly-erupted teeth. J Appl Oral Sci;20(3):352-6. 2012.
10. Sakkas C, Khomenko L, Trachuk I. A comparative study of clinical effectiveness of fissure sealing with and without bonding systems: 3-year results. Eur Arch Paediatr Dent;13(2):73-81. 2013.
11. Lygidakis NA, Dimou G, Stamataki E. Retention of fissure sealants using two different methods of application in teeth with hypomineralised molars (MIH): a 4 year clinical study. Eur Arch Paediatr Dent ;10(4):223-226. 2009.
12. Erbas Unverdi G, Atac SA, Cehreli ZC. Effectiveness of pit and fissure sealants bonded with different adhesive systems: a prospective randomized controlled trial. Clin Oral Investig;21:2235-2243.13. 2017.
13. Bhat PK, Konde S, Raj SN, Kumar NC. Moisture-tolerant resin-based sealant: a boon. Contemp Clin Dent;4(3):343-348. 2013.
14. Boksman L, McConnell RJ, Carson B, McCutcheon-Jones EF. A 2-year clinical evaluation of two pit and fissure sealants placed with and without the use of a bonding agent. Quintessence Int;24: 131-133. 1993.
15. Khare M, Suprabha BS, Shenoy R, Rao A. Evaluation of pit-and-fissure sealants placed with four different bonding protocols: a randomized clinical trial. Int J Paediatr Dent. 2016. Epub ahead of print.
16. Nazar H, Mascarenhas AK, Al-Mutwa S, Aria J, Soparker P. Effectiveness of fissure sealant retention and caries prevention with and without primer and bond. Med Princ Pract;22(1):2-17. 2013.
17. Pinar A, Sepet E, Aren G, Bolukbasi N, Ulukapi H, Turan N. Clinical performance of sealants with and without a bonding agent. Quintessence Int;36(5):355-360. 2005.
18. Bagherian A, Sarraf Shirazi A Sadeghi R. Adhesive systems under fissure sealants: yes or no?: A systematic review and meta-analysis. J Am Dent Assoc;147(6):446-56. 2016.
19. Choi J, Drummond JL, Dooley R, Punwani I, Soh JM. The efficacy of primer on sealant shear bond strength. Pedatr Dent :19(4)286-288. 1997.
20. Hebling J, Feigal RJ. Use of one-bottle adhesive as an intermediate bonding layer to reduce sealant microleakage on saliva-contaminated enamel. Am J Dent:13(4):187-91. 2000.
21. Cehreli ZC, Gungor HC. Quantitative microleakage evaluation of fissure sealants applied with or without a bonding agent: results after four-year water storage in vitro. J Adhes Dent;10(5):379-84. 2008.
22. Tehrani MH, Birjandi N, Nasr E, Shahtuis M. Comparison of microleakage of two materials used as fissure sealants with different methods: an in vitro study. Int J Prev Med;5(2):171-5. 2014.
23. Marks D, Owens BM, Johnson WW. Effect of adhesive agent and fissure morphology on the invitro microleakage and penetrability of pit and fissure sealants. Quintessence Int.;40(9):763.72 2009.
24. Mehrabkhani M, Mazhari F, Sadeghi S, Ebrahimi M. Effects of sealant, viscosity, and bonding agents on microleakage of fissure sealants: An in vitro study. Eur J Dent;9(4):558-63. 2015.
25. Karaman E, Yazici AR, Tuncer D, Firat E, Unluer S, Baseren M. A 48-month clinical evaluation of fissure sealants placed with different adhesive systems. Oper Dent;38(4):369-75. 2013.
26. McMurphy A, Xu X, Fournier S, Cehreli ZC, Sherman K, Tremmel C, Yu Q, Townsend J. Effect of cured versus uncured adhesive inclusion on the microtensile bond strength of sealants. J Dent Child;84(2):58-64. 2017.
27. Fan Y, Townsend J, Wang Y, Lee E, Evans, K, Hender E, Hagan JL, Xu X. Formulation and characterization of antibacterial fluoride-releasing sealant. Pediatr Dent;35(1): E13-8. 2013.
28. Kidd EA. Microleakage in relation to amalgam and composite restorations: A laboratory study. Br Dent J ;141:305-10. 1976.
29. Duangthip D, Lussi A. Microleakage and penetration ability of resin sealant versus bonding system when applied following contamination. Pediatr Dent ;25:505-11. 2003.
30. Futatsuki M1, Kubota K, Yeh YC, Park K, Moss SJ. Early loss of pit and fissure sealant: a clinical and SEM study. J Clin Pediatr Dent;19(2):99-104.1995.