Effect of Bonding Agent on Retention of Different Sealants: An *in Vitro* Study

Moaz H Attar * / Medhat A Abdallah ** / Hussein A Alharthy *** / Omar A El Meligy ****

Objectives: *To investigate the effect of Co-curing versus Staged-curing and No-bonding on retention of different resin-based sealants (RBS).* ***Study design:*** *For shear bond strength (SBS) and microleakage tests, 90 extracted premolars were divided equally into 3 groups (I, II, III). Each group was further subdivided equally into 3 subgroups (a, b, c). No-bonding subgroups did not receive a bonding agent, Staged-curing subgroups received a bonding agent that was cured before sealant application, while Co-curing subgroups received a bonding agent that was cured after sealant application. Seal-it was applied for group I, Helioseal-F for group II and Clinpro for group III. SBS buttons were tested using Instron machine, while microleakage specimens were examined using micro-CT.* ***Results:*** *Clinpro showed the highest SBS values in Staged-curing and No-bonding groups (8.72±2.39, 12.51±3.16) respectively. Staged-curing was significantly greater in SBS values than those for other groups (P<0.05). There was a significant difference in microleakage values of Staged-curing among different RBS (P = 0.003), while there was no significant difference in values of No-bonding and Co-curing among different RBS (P = 0.541, P = 0.521).* ***Conclusions:*** *The use of a bonding agent as Staged-curing was more effective in improving sealant retention than No-bonding and Co-curing.*

Keywords: *Adhesive bonding, Fissure sealant, Microleakage, Micro-CT, Shear bond strength*

INTRODUCTION

Pediatric dentistry guidelines recommend sealing the primary and permanent molars in children and adolescents[1]. The clinical success of fissure sealants (FSs) is highly related to their appropriate application[2]. A dry enamel surface is mandatory to achieve good adhesion[3].

However, the success of sealants is based on some characteristic features, and some of these characteristics include the prevention of ingression of oral fluids and bacteria between the sealant and tooth surface[4].

Few clinical and in-vitro studies advised using adhesive bonding agents under the sealant for improving sealant retention and decreasing microleakage[5-10]. In contrast, other studies reported that a bonding agent applied under the sealant does not improve its retention and does not decrease microleakage[11-16].

Most of microleakage tests methods require the use of a tracer or a dye and cutting the tooth into a series of sections to visualize the extent of staining along the tooth-restoration interface with scanning electron microscopy. The depth of dye penetration along the margin can be measured or graded with a scoring system[17-18]. A shortcoming of these tests is that they provide a two-dimensional (2D) and semiquantitative evaluation of leakage because interfacial staining is visualized on a limited number of tooth slabs, and some tooth structure is inevitably lost with sectioning[19]. Furthermore, tooth sectioning is a time-consuming and destructive procedure that prevents further testing of the specimen.

Moaz H. **Attar**, BDS, MSc, DSc * / Medhat A. **Abdallah**, BDS, MSc, PhD ** / Hussein A. **Alharthy**, BDS, MSc *** / Omar A. **El Meligy**, BDS, MSc, PhD ****

From the Pediatric Dentistry, Faculty of Dentistry, King Abdulaziz University, Jeddah, Kingdom of Saudi Arabia.

* Moaz H. Attar, BDS, MSc, DSc, Assistant Professor.

** Medhat A. Abdallah, BDS, MSc, PhD, Professor.

*** Hussein A. Alharthy, BDS, MSc, Postgraduate Student.

**** Omar A. El Meligy, BDS, MSc, PhD, Professor, Pediatric Dentistry, Faculty of Dentistry, Alexandria University, Alexandria, Egypt.

Corresponding author:
Omar Abd El Sadek El Meligy
Faculty of Dentistry
King Abdulaziz University
P.O. Box: 80209
City: Jeddah 21589, Kingdom of Saudi Arabia
Phone: +966-126952000
Mobile: +966-557521584
Fax: +966-126403316
E-mail: omeligy@kau.edu.sa

Over the last decade, the use of micro-computed tomography (micro-CT) has had considerable development in dental research[20]. Micro-CT is a non-destructive method that, starting from a series of 2D images, produces a three-dimensional (3D) reconstruction of the observed specimen[21]. Lately, the technique has been proposed for the evaluation of marginal leakage in pit and fissure sealing[22-23]. However, micro-CT has been validated in the assessment of marginal leakage at the interface between enamel and FS[23].

At present, there is no standardized method for the in-vitro evaluation of retention of FSs due to different pits and fissures anatomy[24]. Also, there are no studies till date is conclusive regarding the use of bonding agent with Co-curing or Staged-curing when compared to No-bonding in reducing the microleakage and improving the long-term clinical retention of resin-based sealants (RBS).

This research carried out an investigation regarding the effect of bonding agents with Co-curing or Staged-curing when compared to No-bonding on the retention of different RBS in permanent teeth.

The research null hypothesis was: There would be no significant difference in sealants retention with or without bonding.

MATERIALS AND METHOD

Ethical approval was obtained from the Research Ethical Committee at the Faculty of Dentistry, King Abdulaziz University (KAU), Jeddah, Saudi Arabia (Approval no. 089-16).

Sample Size Calculation

The sample size was calculated based on the assumption that the true difference in means in the experimental group and the negative controls equal to 2, with standard deviation equal to 1. The power equals 0.85. The type I error probability associated with this test equal 0.05. The insertion of different values in the G Power 3.0.10 program provided the sample size calculation, which revealed that we will need to study at least seven teeth in each group.

Study Sample

One hundred and eighty extracted sound maxillary or mandibular premolars due to impactions, orthodontic reasons and in case of periodontal disease were collected from different governmental out-patient clinics in Jeddah, Saudi Arabia. Teeth selected were free from caries, restorations, cracks and developmental defects. All teeth were cleaned from debris or blood stains and kept in distilled water at room temperature before the testing procedure.

Randomization

Ninety teeth were divided randomly into 9 equal groups for each test by using Statistical Package for Social Sciences (SPSS) software version 20.0 (Armonk, NY; IBM Corp.) with a uniform random variable generation.

Grouping

For each test, the selected teeth were randomly divided into 3 equal groups of 30 teeth each according to the type of RBS. Group I: sealed with Seal-it (Spident Co., Ltd. In Korea); group II: sealed with Helioseal-F (Ivoclar-Vivadent, NY, USA); and group III: sealed with Clinpro (3M™ ESPE™ Minnesota, USA).

Each group was further subdivided into 3 equal subgroups of 10 teeth each according to the pretreatment procedure. Subgroups Ia, IIa and IIIa: did not receive a bonding agent (No-bonding); subgroups Ib, IIb and IIIb: received a bonding agent that was cured before sealant application (Staged-curing); and subgroups Ic, IIc and IIIc: received a bonding agent that was cured after sealant application (Co-curing).

Sample Preparation

The buccal surface of each tooth was cleaned with fluoride-free prophy. For SBS, the roots were cut 1 mm below cementoenamel junction using low speed saw (TECHCUT 4™, Allied High-Tech Products, Inc. USA). Specimens were embedded in polyester resin while the buccal surface faced upward before testing. The buccal surface of each tooth enamel was minimally ground using sandpaper (grade 600-1200) under cooling to produce a flat surface. For microleakage, standardized rounded cavities were prepared on all buccal surfaces with cavity dimensions: 2.5 mm radius circle and 2 mm depth by using a milling machine (PARASKOP® M Bengo comp., Germany). For both tests, each tooth was etched for 15 seconds according to the manufacturer's recommendation with 35% phosphoric acid etchant and then rinsed and dried for 15 seconds with air-water spray until a chalky white appearance was achieved. The specimens in subgroups Ia, IIa and IIIa did not receive a bonding agent (No-bonding). The specimens in subgroups Ib, IIb and IIIb received two coats of Adper Single Bond (3M ESPE, St. Paul, MN, USA) applied with disposable brush tip then airstream was applied for 2-5 seconds for each coat and then the bond was cured for 10 seconds according to manufacturer's instructions (Staged-curing). The specimens in subgroups Ic, IIc and IIIc received two coats of Adper Single Bond applied with disposable brush tip then air stream was applied for 2-5 seconds for each coat and the bond was not cured until the sealant material was applied (Co-curing). In all subgroups, the sealant material was applied, then cured according to the manufacturer's instructions. After curing of the sealant material, to prevent dehydration, the samples were stored at room temperature in distilled water for 24 hours.

Laboratory Procedure

For SBS test each sample was held parallel to Universal Instron Testing Machine "INSTRON 5944 2KN, MA, USA" at the shearing rod. The crosshead speed of this testing machine was 0.5 mm/minute and results obtained in Megapascals (MPa). The SBS values were measured automatically by using the computer system of the Instron machine. For microleakage test, all teeth were thermocycled using thermocycling machine (SD Mechatronic GmbH comp., Germany) for 1000 cycles between 5-55°C in a water bath with the dwell time of 10 seconds. Teeth apices were sealed with sticky wax to prevent dye penetration. Teeth surfaces were coated with three layers of nail varnish except for the surface of restorations and the surrounding 1mm. Teeth were immersed in 50% silver nitrate for 4 hours at room temperature[21]. All specimens were exposed to the fluorescent light to stabilize the stain. Then they were removed from the dye solution and washed under running water for half an hour. To obtain the X-ray images, each specimen was settled with sticky wax in the specimen holder of the micro-CT system (Model 1172 "Skyscan Kontich, Belgium). To scan all specimens, a setting of 1mm aluminium filter and 100 kV/98mA X-ray source was used. At 180 degrees, each specimen was rotated with a rotation step of 0.40 degrees. The gain was set at 1.0. and the exposure time was 3.7

seconds. The magnification was set at 20, which provided a pixel size of 13.4 lm. All the projected X-ray images had the cone-beam reconstruction using NRecon version 1.6 software. By the use of CT-Analyser V.1.11, axial images were obtained with the cross-sections perpendicular to the bucco-lingual direction of the cavity. To obtain raw data, cross-sections of specimens were collected and converted into 16-bit-mapped image files revealing 2-D images with a resolution of 512x512 pixels. Each sample was randomly given a digital code. The list was kept in a sealed envelope, which ensured that the evaluation would be carried out blindly by one examiner. The examiner was faculty from the Department of Pediatric Dentistry, KAU. The intra-examiner reliability was obtained using SPSS version 21.0 by computing weighted kappa. The kappa value was 0.74 which was in an acceptable range that indicated an acceptable level of consistency. The examiner scored the degree of dye penetration on an ordinal scale ranging from 0 to 3. The examiner had given only the worst score for each sample from both cross and axial sections. The following scoring criteria have been used according to Manhart *et al*[25]: 0 = no dye penetration (Figure 1), 1 = dye penetration to half of the interface length (Figure 2), 2 = dye penetration beyond half of the interface length (Figure 3) and 3 = dye penetration reaches the base, all around the sealant (Figure 4).

Statistical Analysis

Data was analyzed using SPSS version 21.0. The significance level for this analysis was set at α=0.05 and the level of confidence for this analysis was 95%. For SBS test, One-way analysis of variance (ANOVA) followed by Tukey's post-hoc were used for data analysis. For microleakage test, frequency and percentage tests followed by Kruskal-Wallis H test were used for data analysis.

Figure 1. Cross and axial sections showing no dye penetration-Score 0

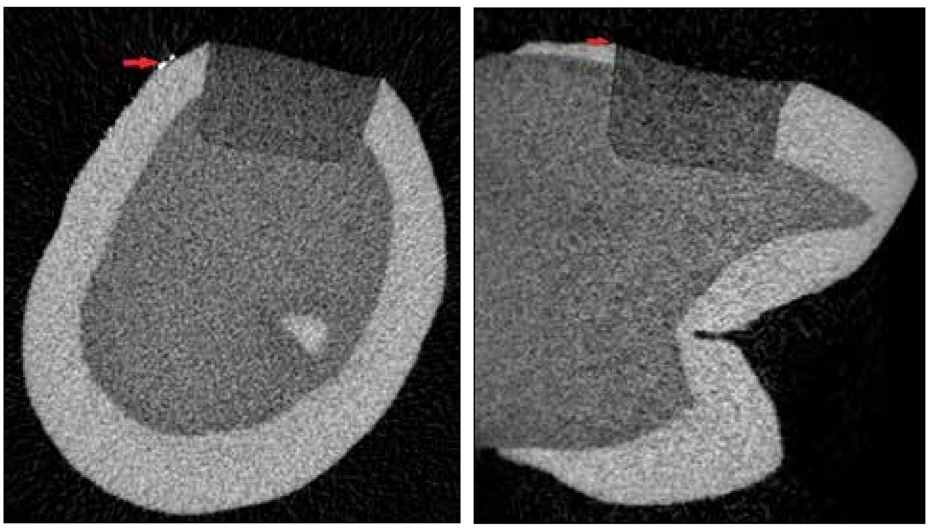


Figure 2. Cross and axial sections showing dye penetration to half of interface length-Score 1

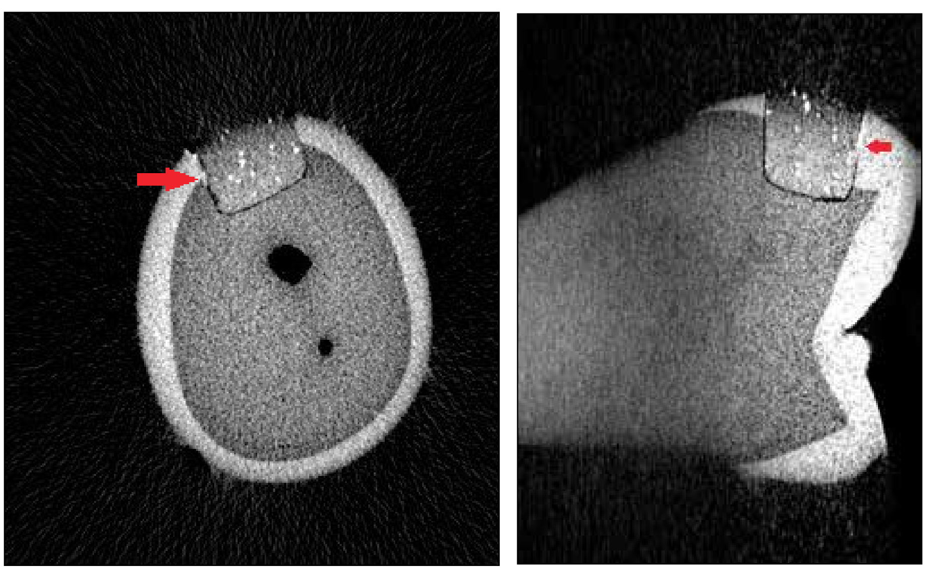


Figure 3. Cross and axial sections showing dye penetration beyond half of the interface length-Score 2

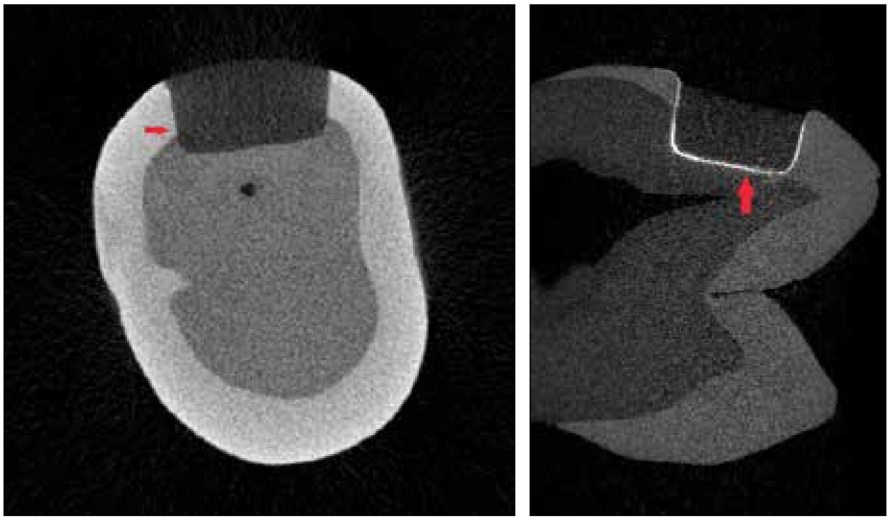
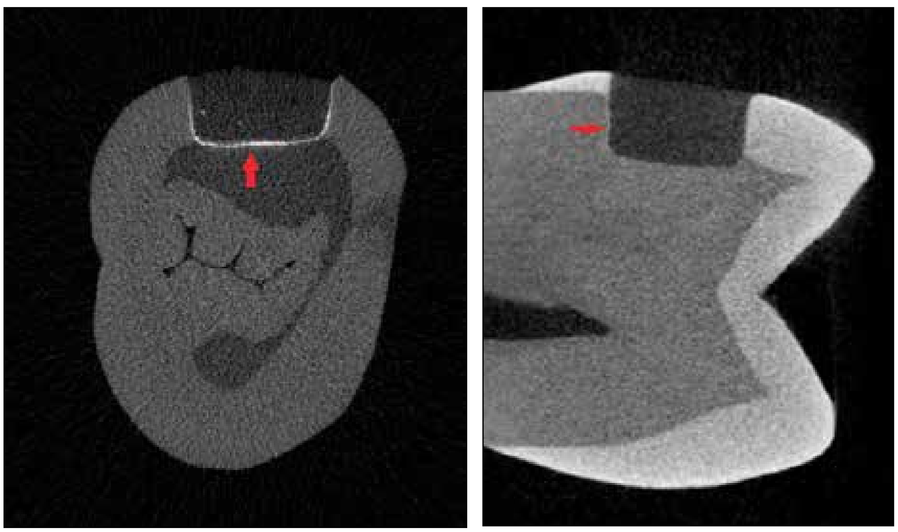


Figure 4. Cross and axial sections showing dye penetration all around the sealant-Score 3



RESULTS

Shear Bond Strength Test

Description of Shear Strength of each group including mean, standard deviation, minimum, maximum values, standard error of mean and range were presented in Table 1. Results of one-way ANOVA revealed that there was significant difference of bond strength of all groups between pretreatment procedures, [Seal-it (F=10.850; P=<0.001), Helioseal-F (F=7.367; P=0.003) and Clinpro (F=16.012; P=<0.001)] (Table 1).

The LSD test indicated that the shear strength of Seal-it and Clinpro interaction with Staged-curing was significantly greater than those for groups of No-Bonding (p=<0.001, p=0.005) and Co-curing (P=0.016 and P=<0.001) respectively. Helioseal-F interaction with Staged-curing was significantly greater than Co-curing (P=0.001) and not significantly greater than the No-Bonding (P=0.187) (Table 2).

Also results revealed that only the No-Bonding subgroups showed significant difference among different sealant materials (F=10.111; P=0.001), Staged-curing (F=1.935; P=0.164) and Co-curing (F=1.245; P=0.304) (Table 3).

The LSD test indicated that the No-Bonding interaction with Seal-it was lower significantly than Helioseal-F and Clinpro (P=0.001 and P=<0.001) respectively. The interaction between all sealant materials was not significantly different based on Staged-curing and Co-curing subgroups (Table 4).

Microleakage Test

Images from micro-CT were eligible for evaluation of microleakage after 3D micro-CT reconstruction.

Frequencies of Microleakage among Study Groups

The outcomes for the microleakage for the scores have shown that most of the Seal-it (No-bonding), Seal-it (Staged-curing) and Seal-it (Co-curing) revealed dye penetration beyond half of the interface. Similarly, the outcomes for Helioseal-F (No-bonding), Helioseal-F (Co-curing), Clinpro (No-bonding) and Clinpro (Co-curing) have shown that most of the teeth revealed dye penetration beyond half of the interface. On the other hand, microleakage outcomes for Helioseal-F (Staged-curing) and Clinpro (Staged-curing) revealed that most of the teeth showed no dye penetration or dye penetration to half of the interface length (Figure 5).

Table 1. Difference in shear strength of various pit and fissure sealants using one-way ANOVA

		Seal-it	Helioseal-F	Clinpro
No-Bonding	Mean	4.5810	8.5340	8.7290
	Std. Deviation	2.44649	2.13683	2.38807
	Minimum	2.02	4.80	6.02
	Maximum	8.92	11.05	13.88
	Std. Error of Mean	0.77365	0.67573	0.75517
	Range	6.90	6.25	7.86
Staged-curing	Mean	11.0460	9.9160	12.5130
	Std. Deviation	3.54676	1.92210	3.16470
	Minimum	4.76	7.46	9.25
	Maximum	16.18	13.15	20.13
	Std. Error of Mean	1.12159	0.60782	1.00077
	Range	11.42	5.69	10.88
Co-curing	Mean	7.4790	6.0460	5.4980
	Std. Deviation	3.22939	2.72072	2.71652
	Minimum	3.37	2.17	2.19
	Maximum	13.61	9.24	11.30
	Std. Error of Mean	1.02122	0.86037	0.85904
	Range	10.24	7.07	9.11
F		10.850	7.367	16.012
P-value		<0.001*	0.003*	<0.001*

ANOVA: analysis of variance

*: Statistically significant P< 0.05

F: F statistic

Table 2. Interaction effect of shear strength for each pit and fissure sealant with different pretreatment procedures by using LSD test

Dependent Variable			Mean Difference	P-value
I	a	b	-6.465	<0.001*
		c	-2.898	0.047*
	b	a	6.465	<0.001*
		c	3.567	0.016*
	c	a	2.898	0.047*
		b	-3.567	0.016*
II	a	b	-1.382	0.187
		c	2.488	0.022*
	b	a	1.382	0.187
		c	3.870	0.001*
	c	a	-2.488	0.022*
		b	-3.870	0.001*
III	a	b	-3.784	0.005*
		c	3.231	0.015*
	b	a	3.784	0.005*
		c	7.015	<0.001*
	c	a	-3.231	0.015*
		b	-7.015	<0.001*

LSD: least significant difference
*: Statistically significant P< 0.05
I: Seal-it
II: Helioseal-F
III: Clinpro
a: No-Bonding
b: Staged-curing
c: Co-curing

Table 3. Difference in shear strength of various pretreatment procedures used with three sealant materials using one-way ANOVA

		Seal-it	Helioseal-F	Clinpro	F	P-value
No-Bonding	Mean	4.5810	8.5340	8.7290		
	Std. Deviation	2.44649	2.13683	2.38807		
	Minimum	2.02	4.80	6.02		
	Maximum	8.92	11.05	13.88	10.111	0.001*
	Std. Error of Mean	0.77365	0.67573	0.75517		
	Range	6.90	6.25	7.86		
Staged-curing	Mean	11.0460	9.9160	12.5130		
	Std. Deviation	3.54676	1.92210	3.16470		
	Minimum	4.76	7.46	9.25		
	Maximum	16.18	13.15	20.13	1.935	0.164
	Std. Error of Mean	1.12159	0.60782	1.00077		
	Range	11.42	5.69	10.88		
Co-curing	Mean	7.4790	6.0460	5.4980		
	Std. Deviation	3.22939	2.72072	2.71652		
	Minimum	3.37	2.17	2.19		
	Maximum	13.61	9.24	11.30	1.245	0.304
	Std. Error of Mean	1.02122	0.86037	0.85904		
	Range	10.24	7.07	9.11		

ANOVA: analysis of variance

*: Statistically significant P< 0.05

F: F statistic

Table 4. Interaction effect of shear strength for each pretreatment procedure with different pit and fissure sealants by using LSD test

Dependent Variable			Mean Difference	P-value
a	I	II	-3.953	0.001*
		III	-4.148	<0.001*
	II	I	3.953	0.001*
		III	-0.195	0.853
	III	I	4.148	<0.001*
		II	0.195	0.853
b	I	II	1.130	0.401
		III	-1.467	0.278
	II	I	-1.130	0.401
		III	-2.597	0.060
	III	I	1.467	0.278
		II	2.597	0.060
c	I	II	1.433	0.279
		III	1.981	0.138
	II	I	-1.433	0.279
		III	0.548	0.676
	III	I	-1.981	0.138
		II	-0.548	0.676

LSD: least significant difference
*: Statistically significant P< 0.05
I: Seal-it
II: Helioseal-F
III: Clinpro
a: No-Bonding
b: Staged-curing
c: Co-curing

Comparisons of Microleakage among Study Groups by Kruskal-Wallis H Test

The outcomes obtained for Kruskal-Wallis H test showed that there was a statistically significant difference in the values of Staged-curing among different materials (P = 0.003). Also, there was no statistically significant difference in the values of No-bonding and Co-curing among different materials (P = 0.541, P = 0.521) (Table 5). Further evaluation revealed that the values for the Helioseal-F and Clinpro were statistically significant (P < 0.001, P < 0.001) with lower microleakage of Staged-curing subgroups mean rank of 7.10 and 5.50 respectively. Seal-it values with Staged-curing subgroup showed insignificant lower microleakage mean rank of 12.60 (Table 6).

DISCUSSION

This study compared three different RBS materials on pretreated enamel either with No-bonding, Staged-curing or Co-curing in permanent teeth by measuring the SBS and microleakage. Based on the results of this study, the null hypothesis was rejected because there was a significant difference in sealant retention with and without bonding.

According to our knowledge, this study was the first to assess the SBS of "Seal-it" material.

Bond strength was considered to be tested as it can reflect the retention and longevity of these sealants[26]. Evaluating the bond strength utilizing the SBS test has advantages such as the balanced distribution of stress, evaluation of smaller surfaces, reducing the effect of enamel defects and recognition of even small differences in bond strength. The reliability of this method was previously confirmed by the work of Placido *et al*[27].

Etemadi *et al*[28] assessed the reproducibility of a previously established cavity preparation design which should be manually reproduced by specialists and found a great difference in the preparations

Figure 5. Percentages of microleakage scores among study groups

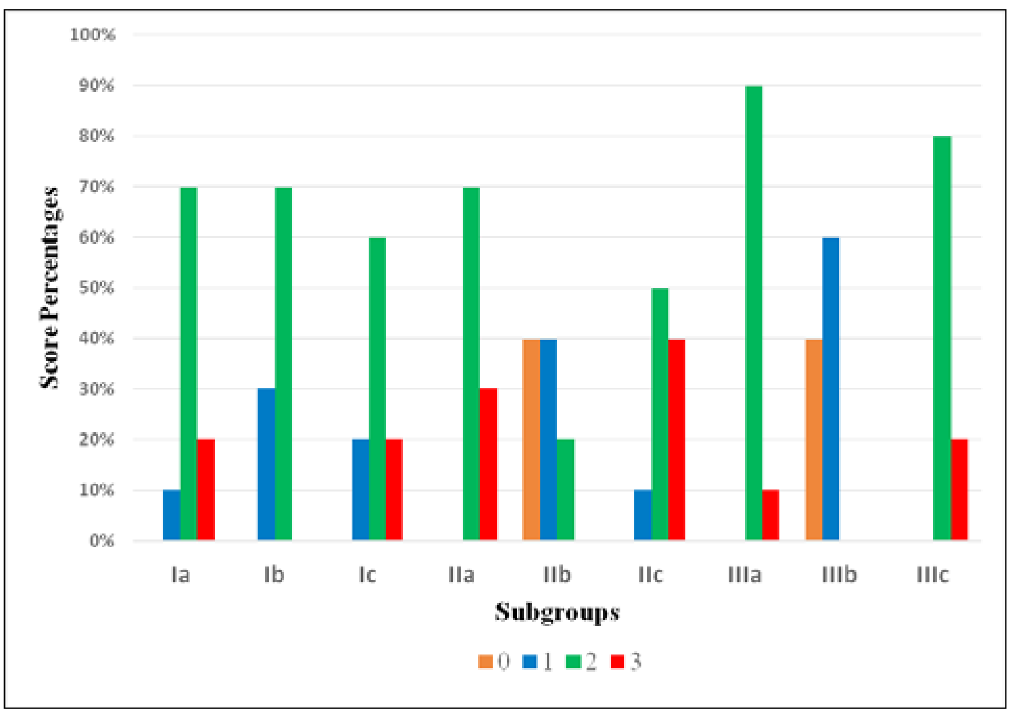


Table 5. Mean ranks and chi-squares of different pretreatment procedure groups using Kruskal-Wallis H test

Subgroups	Mean Rank	Subgroups	Mean Rank	Subgroups	Mean Rank
Seal-it (No-bonding)	14.70	**Seal-it (Staged-curing)**	22.70	**Seal-it (Co-curing)**	13.50
Helioseal-F (No-bonding)	17.35	**Helioseal-F (Staged-curing)**	13.00	**Helioseal-F (Co-curing)**	17.30
Clinpro (No-bonding)	14.45	**Clinpro (Staged-curing)**	10.80	**Clinpro (Co-curing)**	15.70
Chi-Square	1.230	Chi-Square	11.842	**Chi-Square**	1.292
P-value	0.541	P-value	0.003*	P-value	0.524

* Statistically significant at P < 0.05.

Table 6. Mean ranks and chi-squares of different pit and fissure sealant groups using Kruskal-Wallis H test

Subgroups	Mean Rank	Subgroups	Mean Rank	Subgroups	Mean Rank
Seal-it (No-bonding)	17.60	**Helioseal-F (No-bonding)**	19.65	**Clinpro (No-bonding)**	20.00
Seal-it (Staged-curing)	12.60	**Helioseal-F (Staged-curing)**	7.10	**Clinpro (Staged-curing)**	5.50
Seal-it (Co-curing)	16.30	**Helioseal-F (Co-curing)**	19.75	**Clinpro (Co-curing)**	21.00
Chi-Square	2.502	**Chi-Square**	15.526	**Chi-Square**	24.047
P-value	0.286	**P-value**	< 0.001*	**P-value**	< 0.001*

* Statistically significant at P < 0.05.

made by different individuals. In our study, the buccal surfaces received standardized cavities done with the milling machine with standardized depth by using machine digital measurement and standardized width by using bur size without interference by the operator to avoid differences in the preparations. Also, we used buccal surface modelling instead of fissure modelling to standardize our study and to avoid differences in pits and fissures anatomy that could lead to bias of results.

In the current study, we applied the manufacturer's instructions during etching, bonding, sealant application and curing except in subgroups of Co-curing. We studied Co-curing procedure to evaluate the effectiveness in the improvement of sealant retention and to shorten the working time of placing both a bonding agent and sealant in permanent teeth, especially in uncooperative children. In the literature, the idea of using adhesives with sealant on permanent teeth with one curing procedure is not yet firmly studied and manufacturers may take this concept in consideration for their products and work on it.

In this study, to standardize our procedure, we used one type of fifth-generation bonding agent to evaluate its effect on the retention of three different sealant materials since using more than one type of a bonding agent may lead to bias in the concluded results. Among all generations of adhesive bonding, the fifth-generation is considered the best. Furthermore, all samples were thermocycled then stained under the same conditions. Scanning was done by one experienced technician and microleakage evaluation was done by one examiner blindly to avoid bias and methodological errors.

The three sealant materials were selected based on their different compositions. Clinpro is an unfilled RBS, Helioseal-F is a fluoride releasing, filled RBS and Seal-it is filled RBS and relatively new in the market.

In the current study, scoring assessment was used because leakage assessment by the use of computer software is dependent upon the density of the sealant and the bonding agent, as scattering of X-rays from the tooth surface creates various degrees of 'noise' in the background of the scan and upon resolution which is determined by the distance of the tooth to the X-ray source. For example, a low-density dental material may be difficult to distinguish from the 'background noise' and also, perhaps, from the enamel. In our study, we used an assessment of multiple surface scoring methods because assessing a single section of the tooth is not representative because dye penetration varies from one area to another[29]. We used 3D to evaluate microleakage through all samples, this agrees with Raskin *et al* [19] who recommended the utilization of three sections for each restoration to accurately evaluate leakage. Also, Gale and Darvell[30] stated that 3D techniques revealed markedly greater microleakage than 2D assessments.

In contrast to our findings, Boksman *et al*[31] found that the use of bonding agent before sealant application did not increase the retention. The results of this study showed the highest results of shear strength were detected in the Staged-curing subgroups, although the difference in SBS values was insignificant only between the Staged-curing and No-Bonding with Helioseal-F groups. This could be explained by placing the intermediate bonding layer between etched enamel and sealant with significant retention that created strong micromechanical interlocking. In addition, the bonding layer may be affected by higher fluoride content of Helioseal-F that lead to no significant difference between No-Bonding and Staged-curing. Another possible explanation is use of different generations of bonding agents.

On the other hand, our findings agreed with research conducted by Feigal *et al*[8] on the effect of bonding agent on sealant retention, they showed that the bonding agent was effective in sealant retention. This could be explained by the created strong micromechanical interlocking formed by the easy flow of the bonding agent.

Pushpalatha *et al*[32] found out that Clinpro (unfilled sealant) showed better SBS in comparison to Helioseal-F (filled sealant). This agrees with outcomes of this study, as we found Clinpro showed the highest retention with all pretreatment procedures except Co-curing and this could be explained by the low viscosity of the unfilled Clinpro sealant. On the other hand, Co-curing may affect the flow of Clinpro causing lower retention than the other two pretreatment methods.

 doi 10.17796/1053-4625-45.3.6

Additionally, the results from the comparative study of Asselin *et al*[6] showed that the bond strength of bonding and self-etching adhesive groups were higher than the group without bonding. In another study by Ahuja and Ahuja[5] enamel SBS was evaluated through these groups: Single bond, Clear fill protect, Clear fill SE bond, Admira bond and no bond application as the control group. They concluded that Single bond has the highest SBS. This is also in agreement with our study.

For all SBS values, the No-Bonding subgroups revealed a significant difference of retention. Higher retention was recorded for Clinpro followed by Helioseal-F then Seal-it. This agrees with the study of Al-Sarheed *et al*[33] who find out that Visio-Seal had significantly lower bond strength than all 3 other materials using the etchant system and this could be explained by the viscosity factor, in which porosities of the etched surface were filled more with sealant material which has lower viscosity.

A study on retention of FS with or without bonding agents by Pinar *et al*[15] concluded that the use of bonding agents as an intermediary layer between enamel and sealant did not affect sealant success during 24 months. Also, Mascarenhas *et al*[34] concluded that the use of bonding material with sealants does not produce any significant outcomes. However, these results disagree with this study and the possible explanation may due to using different types of sealant materials and bonding agents.

Based on the outcomes of this study, a significantly lower rank of microleakage was with Staged-curing subgroups in both Clinpro and Helioseal-F, while Staged-curing with Seal-it revealed insignificant the lowest rank of microleakage than all other pretreatment procedures. This agrees with Askarizadeh *et al*[35] who found that the reduction of microleakage was due to the placement of sealant with bonding. In addition, Tirali *et al*[10] concluded that pre-treatment adhesive procedures showed lower microleakage than acid etch thus justifying our study results. This may be due to the intermediate bonding layer that created strong micromechanical interlocking between the etched enamel and the sealant which decreased leakage between the tooth structure and the FS.

Furthermore, Cehreli and Gungor[36] used the image analysis toolkit for evaluating the microleakage quantitatively. Regardless of the storage term, the use of etch and rinse adhesives resulted in significantly less microleakage compared to that achieved with self-etching adhesives or acid etching alone. The outcomes obtained from the present research study also demonstrated that the use of adhesive and bonding material decreased the rate of microleakage to some extent. However, they concluded that after four years, sealants placed without a prior bonding agent revealed an enormous amount of leakage and this agrees with our study.

In our study, Co-curing showed the highest microleakage between pretreatment procedure groups. Birlbauer *et al*[37] used materials of three different formulations of an experimental fissure primer (EFP) that was applied without using phosphoric acid etching (EFP-1/EFP-2/EFP-3) and included one control group with sealant application after 30 seconds of acid etching. They concluded that microleakage was significantly lower in the control group than in EFPs groups. This could be due to that Co-curing and EFPs did not form a strong bond between the tooth structure and the sealant material as the conventional etching or the addition of a bonding agent.

This study has some limitations including the relatively long time, which is needed to collect sound permanent teeth. Also, interpretation of these results should consider the limitations of this in-vitro study when compared to clinical trials. In addition, the use of buccal surface modelling instead of fissure modelling could have affected the results. There are clear differences between fissure surface and flat ground buccal enamel surface, such as the presence of aprismatic enamel in fissures and differences in configuration factors.

CONCLUSIONS

The use of a bonding agent as Staged-curing before placement of a RBS in permanent teeth was more effective in increasing SBS and decreasing microleakage than No-bonding and Co-curing.

ACKNOWLEDGEMENTS

This project was funded by the Deanship of Scientific Research (DSR) at King Abdulaziz University, Jeddah, Kingdom of Saudi Arabia under grant no. G-25-165-38. The authors, therefore acknowledge with thanks DSR for technical and financial support.

REFERENCES

1. Wright JT, Crall JJ, Fontana M, et al. Evidence-based clinical practice guideline for the use of pit-and-fissure sealants: a report of the American Dental Association and the American Academy of Pediatric Dentistry. J Am Dent Assoc, 147(8): 672-682, 2016.
2. Barroso JM, Torres CP, Lessa FCR, Pécora JD, Palma-Dibb RG, Borsatto MC. Shear bond strength of pit-and-fissure sealants to saliva-contaminated and noncontaminated enamel. J Dent Child, 72(3): 95-99, 2005.
3. Tulunoglu O, Bodur H, Uctasli M, Alacam A. The effect of bonding agents on the microleakage and bond strength of sealant in primary teeth. J Oral Rehabil, 26(5): 436-441, 1999.
4. 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.
5. Ahuja K, Ahuja T. In Vitro Evaluation Of The Enamel Shear Bond Strength Of A Resin And Ormocer Based Sealant, Pretreated With An Antibacterial, A Total Etch And A Self-etch Adhesive. Indian J Dent Sci, 5(4): 12-15, 2013.
6. Asselin ME, Sitbon Y, Fortin D, Abelardo L, Rompre PH. Bond strength of a sealant to permanent enamel: evaluation of 3 application protocols. Pediatr Dent, 31(4): 323-328, 2009.
7. Dhillon J, Pathak A. Comparative evaluation of shear bond strength of three pit and fissure sealants using conventional etch or self-etching primer. J Indian Soc Pedod Prev Dent, 30(4): 288-292, 2012.
8. Feigal R, 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.
9. Tandon V, Arora V, Yadav V, et al. Concept of probiotics in dentistry. Int J Dent Med Res, 1(6): 206-209, 2015.
10. Tirali RE, Celik C, Arhun N, Berk G, Cehreli SB. Effect of laser and air abrasion pretreatment on the microleakage of a fissure sealant applied with conventional and self etch adhesives. J Clin Pediatr Dent, 37(3): 281-288, 2013.
11. Aman N, Khan FR, Salim A, Farid H. A randomized control clinical trial of fissure sealant retention: Self etch adhesive versus total etch adhesive. J Conserv Dent, 18(1): 20-24, 2015.
12. Biria M, Ghasemi A, Torabzadeh H, Shisheeian A, Baghban AA. Assessment of microshear bond strength: self-etching sealant versus conventional sealant. J Dent (Tehran, Iran), 11(2): 137-142, 2014.
13. Jacker-Guhr S, Ibarra G, Oppermann L, Lührs A-K, Rahman A, Geurtsen W. Evaluation of microleakage in class V composite restorations using dye penetration and micro-CT. Clin Oral Investig, 20(7): 1709-1718, 2016.
14. Lucena C, López JM, Abalos C, Robles V, Pulgar R. Statistical errors in microleakage studies in operative dentistry. A survey of the literature 2001-2009. Eur J Oral Sci, 119(6): 504-510, 2011.
15. Pinar A, Sepet E, Aren G, Bölükbaşı N, Ulukapı H, Turan N. Clinical performance of sealants with and without a bonding agent. Quintessence Int, 36(5): 355-360, 2005.
16. Schuldt C, Birlbauer S, Pitchika V, et al. Shear bond strength and microleakage of a new self-etching/self-adhesive pit and fissure sealant. J Adhes Dent, 17(6): 491-497, 2015.
17. Mali P, Deshpande S, Singh A. Microleakage of restorative materials: an in vitro study. J Indian Soc Pedod Prev Dent, 24(1): 15-18, 2006.
18. Raskin A, D'Hoore W, Gonthier S, Degrange M, Déjou J. Reliability of in vitro microleakage tests: A literature review. J Adhes Dent, 3(4): 295-308, 2001.
19. Raskin A, Tassery H, D Hoore W, et al. Influence of the number of sections on reliability of in vitro microleakage evaluations. Am J Dent, 16(3): 207-210, 2003.
20. Swain MV, Xue J. State of the art of micro-CT applications in dental research. Int J Oral Sci, 1(4): 177-188, 2009.
21. De Santis R, Mollica F, Prisco D, Rengo S, Ambrosio L, Nicolais L. A 3D analysis of mechanically stressed dentin-adhesive-composite interfaces using X-ray micro-CT. Biomaterials, 26(3): 257-270, 2005.
22. Chen X, Cuijpers V, Fan M, Frencken J. Marginal leakage of two newer glass-ionomer-based sealant materials assessed using micro-CT. J Dent, 38(9): 731-735, 2010.
23. Chen X, Cuijpers V, Fan M, Frencken J. Validation of micro-CT against the section method regarding the assessment of marginal leakage of sealants. Aust Dent J, 57(2): 196-199, 2012.
24. Ak AT, Alpoz AR. Effect of saliva contamination on microleakage of three different pit and fissure sealants. Eur J Paediatr Dent, 1: 193-96, 2010.
25. Manhart J, Chen HY, Mehl A, Weber K, Hickel R. Marginal quality and microleakage of adhesive class V restorations. J Dent, 29(2): 123-130, 2001.
26. Kühnisch J, Bedir A, Lo Y-F, et al. Meta-analysis of the longevity of commonly used pit and fissure sealant materials. Dent Mater, xxx.e1-xxx.e11, 2020.
27. Placido E, Meira JB, Lima RG, Muench A, de Souza RM, Ballester RY. Shear versus micro-shear bond strength test: a finite element stress analysis. Dent Mater, 23(9): 1086-1092, 2007.
28. Etemadi S, Smales R, Drummond P, Goodhart J. Assessment of tooth preparation designs for posterior resin-bonded porcelain restorations. J Oral Rehabil, 26(9): 691-697, 1999.
29. Mixson J, Eick J, Chappell R, Tira D, Moore D. Comparison of two-surface and multiple-surface scoring methodologies for in vitro microleakage studies. Dent Mater, 7(3): 191-196, 1991.
30. Gale M, Darvell B. Dentine permeability and tracer tests. J Dent, 27(1): 1-11, 1999.
31. Boksman L, McConnell R, McCutcheon-Jones E. A 2-year clinical evaluation of two pit and fissure sealants placed with and without the use of a bonding agent. Quintessence Int, 24(2): 131-133, 1993.
32. Pushpalatha H, Ravichandra K, Koya Srikanth GD, Done V, Krishna KB, Patil V. Comparative evaluation of Shear bond strength of different Pit and fissure Sealants in Primary and Permanent teeth-An In-Vitro Study. J Int Oral Health, 6(2):84-89, 2014.
33. Al-Sarheed M. Bond strength of 4 sealants using conventional etch and a self-etching primer. J Dent Child, 73(1): 37-41, 2006.
34. Mascarenhas AK, Nazar H, Al-Mutawaa S, Soparkar P. Effectiveness of primer and bond in sealant retention and caries prevention. Pediatr Dent, 30(1): 25-28, 2008.
35. Askarizadeh N, Norouzi N, Nemati S. The effect of bonding agents on the microleakage of sealant following contamination with saliva. J Indian Soc Pedod Prev Dent, 26(2): 64-66, 2008.
36. 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): 379-384, 2008.
37. Birlbauer S, Chiang ML, Schuldt C, et al. Shear bond strength and microleakage results for three experimental self-etching primer compositions for pit and fissure sealing. Clin Oral Investig, 21(5): 1465-1473, 2017.