An evaluation of three fissure sealants microleakage with presence or absence of bonding agent through time intervals (*In vitro study*)

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ABSTRACT

Background: Pit and fissure sealant have been considered an outstanding adjunct to oral health care in the decrease of occlusal caries onset and low progression. The aims of this in vitro study were to evaluate the marginal microleakage of three different types of fissure sealants (SDI, Tg and tetric N-flow) by time interval, one day and 45 days, in the presence or absence of bonding agent among maxillary and mandibular teeth.

Materials and methods: Seventy two sound human maxillary and mandibular first premolar teeth were collected which were free from obvious carious lesions. The teeth were randomly divided into two main equal groups, group (1) and group (2), each group consists of (36) teeth involving equal numbers of maxillary and mandibular teeth. The first group incubated for one day, the second incubated for (45) days. Each group divided into two subgroup; one of them treated with bonding agent while the other without. Then each subgroup was treated with three different materials which were; Tg sealant (without fluoride) group (A), SDI sealant (containing fluoride) group (B) and Tetric N-flow (flowable composite) group (C). Each one consist of six teeth involving three maxillary and three mandibular. Then dye penetration tested by using methylene blue dye, then the teeth cleaned and sectioned by sectioning device and tested under microscope.

Results, the results had shown that, group (C+) in both incubation periods have no microleakage (score 0), but there was an opposite effect when using bonding agent with sealant materials not containing filler particles that showed a significant increase in the microleakage rate as shown in groups (A+ and B+). The opposite effect was seen also when used sealant materials containing filler particles but without bonding agent that seen in group (C) during both incubation periods that showed significant increasing in microleakage rate. While the effect of fluoride was very clear in decreasing significantly the microleakage rate after (45) days of incubation in both groups that treated with and without bonding agent (groups B and B+). Concerning the anatomical variation, there were no significant changes in most groups regarding the microleakage rate.

Conclusions: Results had shown that the microleakage can be prevented by using of flowable composite containing nanofillers that treated with bonding agent after etching of enamel with 35% phosphoric acid gel.

Keywords: fissure sealants, microleakage, bonding agent, time intervals. (J Bagh Coll Dentistry 2013; 25(1):181-187).

INTRODUCTION

Fissure sealants was considered in this study, because there are different opinions related to the sealant materials in a positive or negative direction and there is a controversy in the uses of sealant materials, also pit and fissure sealant has been considered an important way to oral health care in decreasing of occlusal caries onset and low progression by its sealing ability that prevents accumulation of food remnant in the fissures (which is a nutrient for bacteria) and prevention of bacterial leakage to the weakest point of enamel, thus preventing dental caries [(1)], also the benefit of fluoride within sealant materials, in addition to, another study showed that if the sealant dislodged from its location the remnant of sealant materials may remain intruded within the tag and reduce entrance of food particles and bacteria [(2)], and there were different types of sealant have similar or different sealing ability [(3)].

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Sealing treatment could be considered as a minimal invasive and cost effective treatment, and because there were no previous available Iraqi studies related to fissure sealants, so this study was conducted.

MATERIALS AND METHODS

Seventy two sound human maxillary and mandibular first premolar teeth were collected which were extracted for orthodontic purpose of young age ranged between fifteen to twenty years old of age. Teeth should have fully developed roots, free from obvious carious lesions, morphological defects, restorations, and with deep pits and fissures that are typically indicated for sealant placement.The selected (72) teeth were kept in (0.1%) thymol solution to prevent microbial growth after cleaning of all teeth from any remnant. Thenconstruction of the acrylic block was done by mounting each tooth in acrylic block after that, the occlusal surfaces were cleaned with a disposable prophylaxis brush with tapered end by using a low speed contra angle hand piece (5000 RPM) for ten seconds.

The teeth were randomly divided into two main equal groups; group (1) and group (2), each group consisting of (36) teeth involving (18) maxillary first premolar teeth and (18) mandibular. The first group was incubated for one day, while the second incubated for (45) days. Each group was divided into (2) subgroups, each one consisting of (18) teeth involving equal number of maxillary and mandibular teeth. One group was treated with bonding agent, whiles the other group without. Then each group was subdivided into (3) subgroups depending on three different sealant materials used which are; SDI (containing fluoride) sealant; Tetric N-flow (flowable composite), and Tg (without fluoride) sealant. Each subgroup consists of (6) teeth involving (3) maxillary first premolar and (3) mandibular. Before starting the material application technique, a constant distance between tooth surface and source of washing water and drying air from triple syringe should be provided for standardization purpose. The distance between the tooth and light curing source, also it is very important to be adjusted for standardization purpose.No invasive technique was used prior to sealant placement. The occlusal surfaces were cleaned then the enamel in the area of pits and fissures (occlusal surface) was treated with different techniques and sealed with different types of resin based sealant (Table 1).

Table 1: Different treatment groups.

Groups	Sealant materials	Conditioning technique
A	Tg (without fluoride)	Acid etch
A+	Tg	Acid etch + bonding agent
B	SDI (with fluoride)	Acid etch
B+	SDI	Acid etch + bonding agent
C	Tetric N-Flow (with fillers)	Acid etch
C+	Tetric N-Flow	Acid etch + bonding agent

In group A, the occlusal fissures were etched with 35 percent phosphoric acid gel etchant (SDI supper etch , Australia) for (15 to 20) seconds, flushed with an air-water spray (oil-free air and deionized distilled water) for (5) seconds until no traces of etchant gel color could be visible on the surface, then dried by air syringe for (3) seconds. The occlusal surface becomes ready for sealant placement. In group A+, the same steps of etching in group A was applied to group A+, but after etching, the bonding agent (3M ESPS, fifth generation) was applied to the etched occlusal surface with a disposable brush, blowing gently with air from an air syringe for (2) seconds for thinning the adhesive and waiting for (2) seconds allowing bonding agent to infiltrate into the etched surface fissure. Then curing for (20) seconds with visible light cure unit (Astralis 3, vivadent). All steps mentioned in group A and A+ for enamel conditioning techniques were exactly used in other groups B and B+, C and C+ respectively. Before sealant placement, determination of the deepest point of pits and fissures should be done, to make sure that sectioning would be through this point for standardization purpose. The path of microtome sectioning was marked by fine pen on the occlusal surface.The sealant material in group A and A+ was (Tg pit and fissure sealant) that applied immediately after drying of substrate surface, by using of needle tip (supplied within the kit) and a fine probe was used by moving it gently through applied sealant material for better adaptation and air bubble escaping. Then curing of sealant was done for 30 seconds (as manufacture instructions) with visible light cure unit under standardization technique. Two types of aging process were used to accommodate the similar environment subjected to sealant materials within the oral cavity that affect the microleakage.Immediately after completion of sealant applications, the teeth immersed and stored in the artificial saliva which was prepared previously, and the teeth were incubated within a container of artificial saliva at room temperature for a period of one day, this is for (Group 1), while (Group 2) incubated for (45) days. The teeth were subjected to artificial aging by thermocycling in water, the teeth were cycled manually between two water baths [(4)]. One of them maintains the temperature at 52 ± 4 ^{o}C, the other water bath maintain the temperature at 5 ±2 ^{o}C. The immersion time was (30) seconds ± 5 in each bath[(5)], and the number of cycles employed was 15 cycles in each day (15 cycles for Group 1 and 675 cycles for Group 2). After thermo cycling procedure, the teeth were prepared for dye penetration. The specimens' apices were covered with compound wax (if any apex was exposed). After that, each tooth was painted with two layers of nail varnish except the occlusal surface. Then they were immersed in methylene blue dye for four hours[(6)], after that the teeth were rinsed thoroughly under tap water, varnishes were then scrubbed and the teeth allowed to dry, to be prepared for the next step which is sectioning of teeth.The crowns of teeth were sectioned longitudinally in a buccolingual direction through an imaginary line between two

points determined previously. The sectioning was done by using hard tissue microtome (struersMinitom). Each section was examined under electronic microscope, images were captured. A ranked scale method was used to measure microleakage, the rank explained in Table 2 [7].

Table 2: Microleakage scores

Score	Criteria
0	No dye penetration
1	Dye penetration into the Occlusal third of the enamel-sealant interface.
2	Dye penetration into the middle third of the interface.
3	Dye penetration in to the apical third of the interface.

RESULTS

The dye penetration represent the microleakage which was presented by scores for all groups that incubated for one day and for (45) days (Table 3 and 4 respectively).

Table 3: Microleakage scores for all the tested groups incubated for one day and (1) day.

	Micro leakage scores							
	group	**No. of teeth**	**A**	**A+**	**B**	**B+**	**C**	**C+**
ONE DAY	**Maxill.Teeth**	1	0	0	1	3	1	0
		2	1	1	2	2	1	0
		3	0	0	2	3	1	0
	Mandi. Teeth	1	1	0	1	3	1	0
		2	2	1	1	3	1	0
		3	1	0	2	3	1	0

Table 4: Microleakage scores for all thetested groups incubated for one day and (45) days.

45 DAYS	**group**	**No. of teeth**	**A**	**A+**	**B**	**B+**	**C**	**C+**
	Maxill.Teeth	**1**	**0**	**3**	**1**	**1**	**3**	**0**
		2	**0**	**2**	**1**	**0**	**3**	**0**
		3	**1**	**2**	**0**	**1**	**2**	**0**
	Mandi. Teeth	**1**	**1**	**1**	**0**	**0**	**3**	**0**
		2	**1**	**1**	**0**	**0**	**2**	**0**
		3	**1**	**1**	**1**	**0**	**2**	**0**

The frequency of each group was shown in (Fig. 1) and (Fig. 2). Most frequency of score (0) appear in group (C+) during both incubated periods (in this group the type of material was tetric N-flow which is flowable composite treated with bonding agent).

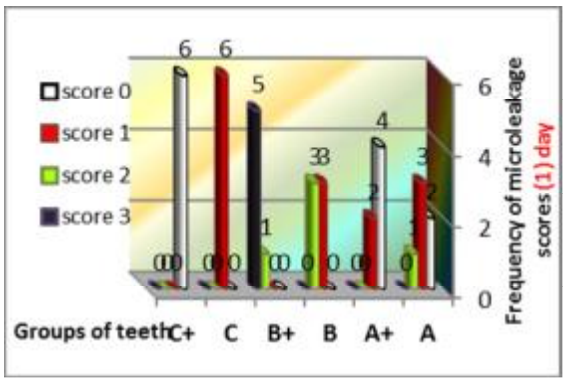


Figure 1: Frequency of microleakage scores among groupsof teeth incubated for one day.

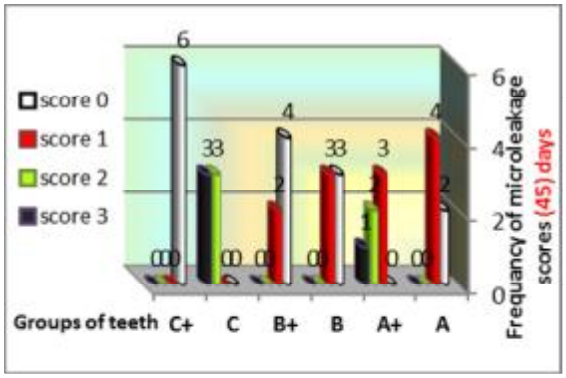


Figure 2: Frequency of microleakage scores among groups of teeth incubated for (45) days.

The results of dye penetration measurement were expressed by descriptive statistic, the lowest mean of microleakage appear in group (C+) in both incubated periods which is (0.00).The results of microleakage means of each group can be compared with other groups to be easier by presenting the percentage of means which are shown in (Fig. 3) and (Fig. 4).The comparisons of microleakage means between two different conditioning techniques are presented in Table (5). Regarding groups incubated for one day, it was found that the microleakage mean value was significantly higher in group (B+) than group (B) for one day incubation, also it was significantly higher in group (A+) than group (A), and it was significantly higher in group (C) than group (C+) regarding groups incubated for (45) days. ANOVA was used to compare between groups treated with different types of materials through

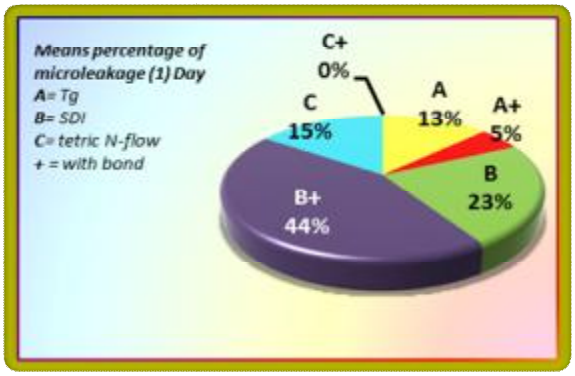


Figure 3: Means percentage of microleakage among groups of teeth incubated for one day.

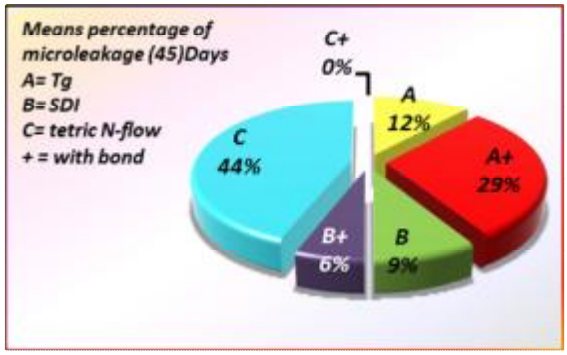


Figure 4: Means percentage of microleakage among groups of teeth incubated for (45) days.

Table 5: Microleakage among groups of teeth with and without using of bonding agent through the same type ofmaterial and the same period of incubation.

Incubation period	Bonding agent	Studied groups	No.	mean	±SD	t-value	Comparison of significant		
							df	P-value	Sig
(1) day	With	A+	6	0.33	0.516	-1.342	10	0.209	NS
	Without	A	6	0.83	0.753				
	With	B+	6	2.83	0.408	4.781	10	0.001	HS
	Without	B	6	1.50	0.548				
	With	C+	6	0.00	0.000	non	10	non	
	Without	C	6	1.00	0.000				
(45) days	With	A+	6	1.67	0.816	2.535	10	0.030	S
	Without	A	6	0.67	0.516				
	With	B+	6	0.33	0.516	-0.542	10	0.599	NS
	Without	B	6	0.50	0.548				
	With	C+	6	0.00	0.000	-11.180	10	0.000	HS
	Without	C	6	2.50	0.548				

the same conditioning technique and the same incubation period as presented in Table (6),within the groups incubated for one day and treated with bonding agent, the three different materials showed a highly significant difference regarding the microleakage means. Least significant difference (LSD) test was needed to show where the significance had occurred between groups that presented in Table (7). Results had shown highly significant differences within group (A+ and B+), (B+ and C+) for one day incubation, (A+ and C+), (A and C) and (B and C) for (45) days incubation. Concerning the comparisons between twodifferent incubation periods among the same type of sealant materials and the same conditioning technique as presented in Table (8). The results showed that groups (B+) and (B) that incubated for one day had microleakage mean values which weresignificantly higher than that in groups incubated for (45) days. To compare between whole maxillary and mandibular teeth treated with the same types of materials, the same conditioning techniques and the same incubation periods, as presented in Table (9).

Table 6: Analysis of variancetest comparison among all groups.

Incubation period	Bonding agent	groupsStudied			F	df	P-value	Sig.
(1)day	With	A+	B+	C+	99.615	2	0.000	HS
	Without	A	B	C	2.500	2	0.116	NS
(45)days	With	A+	B+	C+	15.000	2	0.000	HS
	Without	A	B	C	25.577	2	0.000	HS

Table 7: Least significant difference among studied groups treated with different types of materials during the same period of incubation and the same conditioning technique.

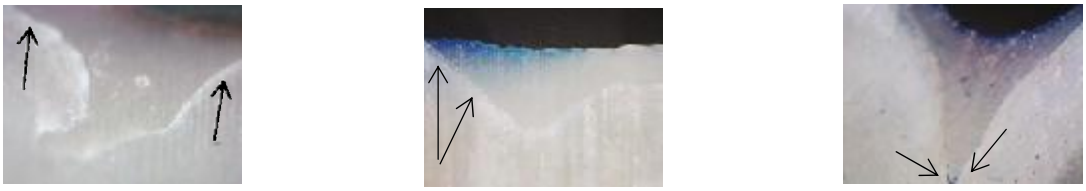
Incubation period	Bonding agent	Studied groups	LSD		
			Mean difference	P-value	Sig.
(1)day	With	A+ - B+	-2.500	0.000	HS
		A+ - C+	0.333	0.150	NS
		B+ - C+	2.833	0.000	HS
(45)days	With	A+ – B+	1.333	0.01	S
		A+ – C+	1.667	0.000	HS
		B+ – C+	0.333	0.317	NS
	Without	A – B	0.167	0.599	NS
		A – C	-1.833	0.000	HS
		B – C	-2.000	0.000	HS

Table 8: Microleakage among groups of teeth with different incubation periods through the same material and the same conditioning technique.

Bonding agent	Incubation periods	Studies groups	No.	mean	±SD	t-value	Comparison of significant		
							df	P-value	Sig.
With	(1) day	*A*+	6	0.33	0.516	-3.381	10	0.007	HS
	(45) days	*A*+	6	1.67	0.816				
	(1) day	*B*+	6	2.83	0.408	9.303	10	0.000	HS
	(45) days	*B*+	6	0.33	0.516				
	(1) day	*C*+	6	0.00	0.000	non	10	non	
	(45) days	*C*+	6	0.00	0.000				
Without	(1) day	*A*	6	0.83	0.753	0.447	10	0.664	NS
	(45) days	*A*	6	0.67	0.516				
	(1) day	*B*	6	1.50	0.548	3.162	10	0.010	S
	(45) days	*B*	6	0.50	0.548				
	(1) day	*C*	6	1.00	0.000	-6.708	10	0.000	HS
	(45) days	*C*	6	2.50	0.548				

Table 9: Microleakage among different types of teeth (maxillary and mandibular first premolars).

Studied groups	No.	mean	±SD	t-value	Comparison of significant		
					df	P-value	Sig.
Maxillary teeth	36	1.06	1.068	0.347	70	0.730	NS
Mandibular teeth	36	0.97	0.971				



(Score 0) (Score 1) (Score 3)

Figure 5: Photograph view of ground sections for different scores (arrows), (x 500).

The results had shown non-significant differences between maxillary and mandibular first premolar teeth regarding the microleakage mean values. The dye penetration and microleakage scores were determined by microscopic examination, and some photographs were taken and presented in Figures (5).

DISCUSSION

The most important result in this study concerning the bonding agent regarding the microleakage reduction; presented by using of flowable composite with bonding agent (group C+) in both times interval, in which there was no any dye penetration (score 0, mean = 0). This

result may be attributed to the effect of bonding agent and the presence of filler particles together.The efficacy of bonding agent on reducing microleakage may be attributed to the combination of both adhesive forces (micromechanical adhesion by tag formation with enamel surface and chemical forces with sealant materials).While the efficacy of filler particles on reducing microleakage may be due to decreasing in the thermal expansion and polymerization shrinkage of sealant materials making it more closely approximate that of natural tooth structure, in addition to that, the fillers help indecreasing the polymerization shrinkage of underneath bonding agent [8, 9].But in group (C) and due to absence of bonding agent, there was an opposite effect of flowable composite on microleakage reduction which was very obvious in group (C) by both time interval. In group (C), the significant elevation of microleakage rate may be due to the presence of filler particles itself; when sealant material applied to the etched enamel surface, the filler particles will interfere with infiltration of the material to the microholes of etched enamel resulting in less penetration and weakened the attachment which can be distorted easily by dimensional changes of material (polymerization and thermal changes) leading to the broking of marginal integrity ending in penetration of dye. Therefore, the microleakage in group (C) was significantly increased with time from less microleakage within group incubated for one day, to obvious increasing in microleakage within group incubated for (45) days due to increasing of thermal dimensional change by effect of thermocycling [10,11].While there was an opposite effect of bonding agent when used with the sealant materials which were free of filler particles by increasing the microleakage mean values significantly in group (B+) more than that of group (B) that incubated for one day, and in group (A+) more than that of group (A) that incubated for (45) days; and this result may be due to the absence of filler particles (which act as a barrier that decreasing the curing light penetration to underneath bonding agent) and may lead to increasing the effect of double polymerization shrinkage of bonding agent that exposed to double curing times, which disturbs the marginal integrity and increase dye penetration [9].Concerning the time interval of sealant materials;when compared the microleakage means of SDI type of sealant material between groups (B), (B+) in both incubation periods; the results showed obvious significant jumping in reduction of microleakage mean values with increasing the time, which may be attributed to the fluoride content of sealant material that precipitate to enamel surface, and because there is a continuous release of fluoride ion from sealant material to the superficial layer of enamel that may obliterate any microgap between the material and enamel within the tags formed leading to intimate contact of material within the tags and become more resistant to dislodgement which occur by thermal dimensional changes through the time and thus decreasing the microleakage, this opinion suggested by other studies[8,12].While the results of group (A) and (A+), in which the sealant material was plane, without filler particles and without fluoride, had shown that the microleakage mean value was increased by the time which was significantly higher in group (A+) incubated for (45) days than group (A+) incubated for one day. This result may be attributed to higher dimensional changes of sealant material through the time (thermal change and polymerization shrinkage), due to absence of filler particles and due to absence of fluoride [10]. Concerning different types of sealant materials; the result showed that group (C+) was the best group that have no dye penetration in both incubation periods. Concerning the maxillary and mandibular teeth, results of this study had shown that both types of teeth exhibit similar marginal sealing ability and further studies were needed to conform this finding to larger sample size.

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