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Clinical and Scanning Electron Microscopic Evaluation of Amorphous Calcium Phosphate Containing Sealant versus Conventional Sealant

Aya Magdy El- Sadany[1], Hatem El Sayed Amin[2], Olfat Mohammed Gaballah[3] , Ahmed Ibrahim El Dosoky[4]

[1](*Pediatric Dentistry, Oral Health and Preventive Department, Faculty of Dentistry - Tanta University, Egypt*)
[2](*Professors andHead of Pediatric Dentistry, Oral Health and Preventive Department Faculty of Dentistry at Tanta University in Egypt*)
[3](*professor of oral biology, Faculty of Dentistry, Tanta University, Egypt Professor and head of oral and maxillofacial surgery and diagnostic sciences. Faculty of dentistry magmaah university, Riyadh KSA*)
[4](*Lecturer at Pediatric Dentistry, Oral Health and Preventive Department, Faculty of Dentistry, Tanta University, Egypt*)

Abstract

Back ground: *Globally, dental caries level has shown a marked decline in the last decades as a result of improved oral hygiene and wide use of fluoride. However, over 80% of dental caries still occur in the occlusal. Therefore, newly smart material has been conducted to act as primary prevention of deep pits and fissures and stimulating the repair of tooth structure such as fissure sealant containing amorphous calcium phosphate (ACP).*

Aim: *This study aimed to assess and compare the clinical effectiveness of amorphous calcium phosphate containing sealant (ACP) versus conventional sealant. To evaluate the penetration and the adaptation ability of the two sealant materials.*

Material and methods*: the study was conducted as a split mouth randomized clinical trial.Sixty sound fully erupted lower first permanent mandibular molars out of 30 children were randomly allocated into two equal groups using a split mouth design. the fissures were sealed with ACP-containing sealant (Aegis) and resin-based sealant (Heliosael). Thirty non-carious upper premolars extracted for orthodontic reasons were used for scanning electron microscopic examination and the teeth were randomly assigned into two equal groups then sealed with two fissure sealant material.*

Result*:Chi- square test revealed that there was no statistically significant difference of sealant retention and caries prevention among groups. T test revealed a non-statistically significant difference between both groups regarding sealant adaptation and penetration.*

Conclusion*: ACP containing sealant was not significantly different from conventional resin-based sealant in retention and caries prevention. Both materials had a remarkable adaptation to the lateral wall of the tooth structure and had a high ability to penetrate into deep pits and fissures.*

Key word*: Aegis, Heliosael, fissure sealant, scanning electron microscope*

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I. Introduction

Pit and fissure caries is still one of the most prevalent oral diseases in which the complex, unpredictable, irregular shape of fissures allow the formation of dental caries. Therefore, different preventive strategies such as oral hygiene and fluoride application have been used. However, these measures decreased the incidence of smooth surface carries with a less effect on occlusal surface caries [1-5].

Fluoride has been documented to be less effective for preventing carious lesions in pit and fissure surfaces compared with smooth surfaces [6, 7]. Therefore, pit and fissure sealants have been developed, as a micromechanically bonded protective layer, to prevent dental caries occurrence in the occlusal stagnation sites of caries susceptible teeth [7-10]. Various researches have indicated that pit-and-fissure sealants are effective in preventing dental caries on an individual level and as a public health measure for high risk populations [9, 11, 12].

The world health organization (WHO) considered that sealing of pits and fissures is one of the most important primary preventive measures in preventing occlusal caries mainly in young permanent molars [12, 13]. Therefore, various sealant materials have been attempted for sealing pits and fissures of occlusal surfaces [14].

Since fissure sealant had been introduced, materials had progressed from biodegradable cyanoacrylate to bisphynolAglycidalmetha acrylate (BIS-GAMA) which resist the biodegradation [11, 15]. After that resin-based sealant is classified according to methods of polymerization, color and filler content [16, 17].

Success of pit and fissure sealant in preventing dental caries is associated with its retention which is based on the penetration ability of the sealant into the complete depths of pits and fissures, and an intimate adaptation to lateral wall of the fissures [18, 19].

Different modifications have been applied to resin sealant materials in order to improve their clinical effectiveness such as longevity, retention and caries prevention. One of these modifications was addition of filler in which filled sealant has greater microhardness and wear resistance than unfilled fissure sealant. Moreover, addition of remeneralizing agent such as fluoride and amorphous calcium phosphate improve the caries prevention of fissure sealant material [2, 5, 20].

Amorphous calcium phosphate (ACP) is the initial solid phase precipitated from highly supersaturated calcium phosphate solution and has the ability to be converted into stable crystalline phase such as apatite[21, 22].Because of excellent biological properties of ACP such as better osteoconductivity than hydroxyapatite (HAP), better biodegradability than tricalcium phosphate and good bioactivity, it is widely used in dentistry[22, 23].

Unlike fluoride, amorphous calcium phosphate containing sealant is considered active smart material because of its ability to release calcium and phosphorous ions only when pH of oral environment becomes acidic. Upon decreasing pH, ACP is broken down releasing saturating levels of Ca2+ and PO4 ions that are responsible for remineralization of tooth structure and maintaining the supersaturation conditions over extended periods of time [24, 25].

Up till now, few studies evaluated the clinical effectiveness of ACP containing sealant [23, 26]. Additionally, no information about its penetration and adaptation under scanning electron microscope is currently available.

Therefore, the present study was conducted to assess and compare the clinical effectiveness of amorphous calcium phosphate containing sealant versus conventional sealant. And to evaluate the penetration and the adaptation ability of the two sealant materials.

II. Materials And Methods

Study design

The present study was carried out as a split mouth randomized clinical trial.

Study setting

The clinical part of this study was conducted in Pedodontic clinic, Faculty of Dentistry, Tanta University and the laboratory work was performed in the scanning electron microscope unit, Faculty of science, Alexandria University.

I- Clinical study

Sample selection

Thirty children aged 6-8 years attending Pedodontic clinic, Faculty of Dentistry, Tanta University were included in this work. Each child had two sound fully erupted lower first permanent molars with deep narrow central fissures and supplemental grooves.

Exclusion criteria: [26, 27]

Children with the following criteria were not included in the study:

- Any restorations or decay in the lower permanent 1st molars.
- Any professionally applied caries preventive measures carried out in the last two years.
- Children with chronic systemic diseases or children with physical or mental handicapped conditions.
- Hypoplastic teeth.

Group assignment:

Sixty first permanent mandibular molars out of 30 children were randomly allocated into two equal groups using a split mouth design.

Group I: The fissures of selected teeth were sealed with amorphous calcium phosphate-containing fissure sealant (Aegis)*.

Group II: The fissures of selected teeth were sealed with resin-based fissure sealant (Heliosael)**.

*Aegis (Bosworth Co. Ltd.USA)

**Heliosael (Ivoclar Viva Dent inc.SchaanLiechtenstein,Switzerland.)

Patients' rights:

Approval for this research was obtained from Research Ethics Committee, Faculty of Dentistry, Tanta University. The informed consents were obtained from children's parents according to the guidelines on human research adopted by the Research Ethics Committee, Faculty of Dentistry, Tanta University.

Clinical procedures [26, 27]

1) Isolation

Complete isolation with rubber dam was assured before sealant placement**.**

2) prophylaxis

The occlusal surfaces of the selected teeth were cleaned with a rotary brush and fluoride-free pumice, then washed with air water spray and dried with oil-free air stream.

3) Surface conditioning

Acid etching was performed using 37% phosphoric acid gel* for thirty seconds [27]. The etchant was gently moved on the occlusal surfaces by soft micro brush to enhance the penetration ability of the etchant into the fissure. Etchant was copiously washed by water for 30 seconds and air dried.

4) sealant application

The sealant was applied starting from the maximum depth of the central fissure up towards the two thirds of cusp height and cured with light source according to the manufacturer's instructions. After the sealants were placed, the rubber dam was removed and the occlusion was checked with a carbon marker. Any premature contact was removed.

Clinical evaluation:

Clinical evaluation was carried out under normal clinical conditions with a dental operating light, mouth mirror and sharp dental explorer. The children were recalled at 1, 3, 6 and 9 months for clinical evaluation of:

1- Sealant retention

Sealant retention was evaluated using Simonsen's criteria as[28]:

- Completely present (C): no sealant ledge was present.
- Partially present (P): part of sealed pit or fissure, or both, was being exposed.
- Completely missing (M): no trace of sealant could be detected.

2- Dental caries

Dental caries was evaluated according to World Health Organization dental caries criteria [29].

II- Scanning electron microscopic examination (SEM)

Thirty non-carious upper premolars extracted for orthodontic reasons were used for scanning electron microscopic examination. Teeth were cleaned using a dental prophylactic cup and then stored in distilled water at 4^{o}c until use.

Group assignment:

The teeth were randomly assigned into two equal groups comprising fifteen teeth each.

Group I: The fissures of selected teeth were sealed with amorphous calcium phosphate-containing fissure sealant (Aegis)*.

Group II: The fissures of selected teeth were sealed with resin-based fissure sealant (Heliosael)*.

Occlusal surfaces of each tooth were etched with 37% phosphoric acid** for 30 seconds, then copiously washed by water and air dried until a frosted appearance of the occlusal surface was seen.

Both sealant materials were manipulated according to the manufacturer's instructions. The sealants were applied from the central fissure up towards the cusps in order to prevent voids, air entrapment or bubbles and light cured.

Specimens' preparation for scanning electron microscope

The roots of each tooth were separated from its crown using diamond disc under water spray at the cement-enamel junction. Then, the crowns were sectioned buccolingually, with a water-cooled diamond saw yielding two sections from each tooth.

The specimens were allowed to dry for 24 hours before subjecting them to gold sputtering. The specimens were mounted on aluminum stubs using double-sided adhesive tape. The mounted surfaces were then coated with a thin layer, 25 nm thicknesses, of pure gold using an ion sputtering unit. The stubs were placed in the vacuum chamber of the SEM unit. The accelerating voltage, angle of tilt and the aperture was adjusted to

*37%phosphoric acid (Ivoclar Viva Dent inc.Schaan Liechtenstein, Switzerland)

*Aegis (Bosworth Co. Ltd.USA)

*Heliosael (Ivoclar Viva Dent inc.SchaanLiechtenstein,Switzerland.)

**37%phosphoric acid (Ivoclar Viva Dent inc.Schaan Liechtenstein, Switzerland) suit the specimen to optimize the quality of the micrograph. The surfaces were scanned and observed on the screen under magnification [30, 31]

Measurement of sealant penetration

The depth of sealant penetration into central of each specimen was measured. The fissure's total depth was estimated by the distance between the most superficial and the deepest points of the central fissure. Then, the percentage of the sealant penetration was calculated through dividing the length of the central fissure filled with the sealing material by its total depth [31].

Measurement of sealant adaptation

The gap width between the sealant and tooth surface was considered as a measurement for sealant adaptation. It was measured under higher magnification by placing the two indicators marks at two extremes of the gaps along the lateral walls and the distance between them was digitally measured. Then the minimum and the maximum width in each specimen was recorded and the mean was calculated [30, 31].

Examiner reliability:

Pit and fissures sealants were applied and evaluated for all cases by only one examiner. Intra-examiner calibration was verified by clinical re-examination of 5 children with pit and fissure sealant with three-weeks interval between examinations. Duplicate measurements of sealant penetration and adaptation of five specimens were also performed to ensure uniform assessment of the variables (Kappa: 0.9).

Statistical analysis

Data was collected, presented and statistically analyzed using SPSS v 22.0 statistical package system [32] Descriptive and inferential statistics using chi square and t test were used at a significance level of 0.05.

III. Results

Table 1 illustrates the clinical evaluation of retention in group I. At 1 month and 3 months follow up periods, sealants were completely present in 90% of cases while 10% of them had been partially loss. At 6 and 9 months follow up periods, 85.71% of sealed teeth were completely present and 14.29% were partially lost. Two patients were lost to follow up at 6 and 9months follow up periods. There were no statistically significant differences among different periods within the group (χ^2 =0.502, **p**=0.918).

While the clinical evaluation of sealant retention in group II resin-based fissure sealant. At 1, 3 and 6 months follow up periods 13.34%, 16.67% and 21.43% of cases were partially lost respectively. At 9 months follow up, 17.86% and 3.57% of sealants were partially lost and completely missing respectively. A sample attrition of two patient occurred at 6 and 9 months follow up. Chi–square test revealed a statistically non-significant difference of the sealant retention rate at different follow up periods. (χ^2 =3.573, **p**=0.734).

The comparison of sealant retention between group I and group II at different follow is demonstrated in Table 3. At 9-months follow, 85.71% and 78.57% of the sealed teeth were completely present among group I and group II respectively. Partial sealant loss in both groups represented 14.29% and 17.86%respectively. Only 3.57% of the sealants were completely missed in group II. The differences did not reach the level of significance (χ^2= 1.58, **P** = 0.45).

Table 1: Comparison of retention rate of group I and group II fissure sealants at different follow up periods

Groups / Follow up Periods	Group I			Group II			χ^2	P value
	Complete present	**Partial loss**	**Complete missing**	**Complete present**	**Partial loss**	**Complete missing**		
1month n =30	27(90%)	3(10%)	0	26(86.66%)	4(13.34%)	0	0.162	0.687
3month n =30	27(90%)	3(10%)	0	25(83.33%)	5(16.67%)	0	0.582	0.445
6month n =28	24(85.71%)	4(14.29%)	0	22(78.57%)	6(21.43%)	0	0.490	0.484
9month n =28	24(85.71%)	4(14.29%)	0	22(78.57%)	5(17.86%)	1(3.57%)	1.585	0.453
χ^2	0.502			3.573				
P value	0.918			0.734				

The difference is significant at the 0.05 level

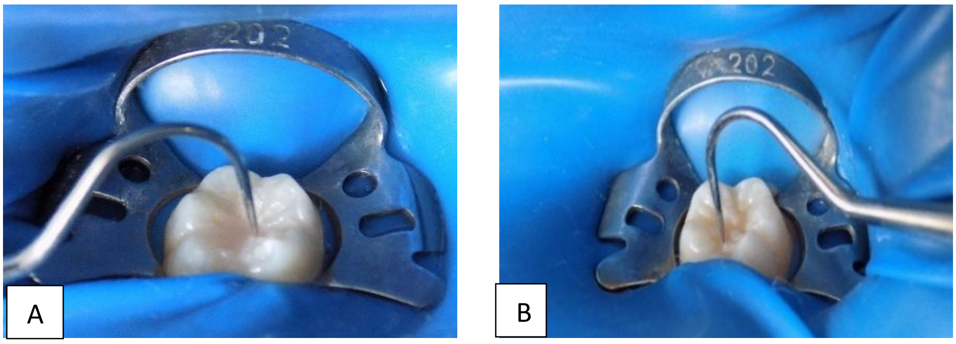

Figure 1: At 9 month (**A)** Group I amorphous calcium phosphate containing sealant showing complete sealant retention of lower right first molar**(B)** Group II resin based sealant of lower left first molar showing Complete sealant retention

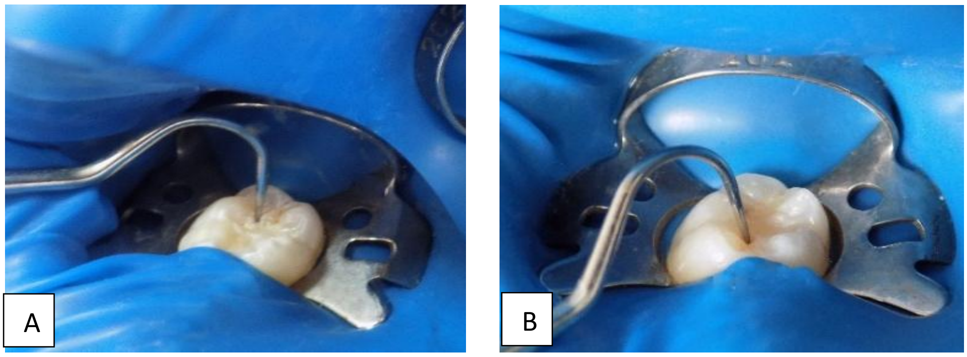

Figure2(A) Group I amorphous calcium phosphate containing sealant of lower left first molar showingPartial sealant loss at9 month**(B)** Group II resin based sealant of lower right first molar showing Partial sealant loss at 9 momths

The caries status in group I and group II at different time periods is illustrated in the Table 4. All cases were caries free among both groups at one month follow up. Three months later, 3.33% of the cases had dental caries in group II. At 6 months and 9 months, 3.57% of the cases become carious in both groups. Chi- square test revealed a non- statistically significant difference in caries among groups. (χ^2= 1, **P** = 0.83).

Table 4 Comparison of caries status in group I and group II at different time periods

Caries status \ Follow up		1month n=30	3months n=30	6months n=28	9months n=28
Group I	Caries present	(0) 0	(0) 0	(1) 3.57%	(1) 3.57%
	Caries absent	(30) 100%	(30) 100%	(27) 96.43%	(27) 96.43%
Group II	Caries present	(0) 0	(1) 3.33%	(1) 3.57%	(1) 3.57%
	Caries absent	(30) 100%	(29) 96.67%	(27) 96.43%	(27) 96.43%
χ^2		1			
P		0.833			

Figure 3: Group II resin-based sealant **A** Caries present at 3months

Scanning electron microscope

Results of sealant penetration and adaptation

Table 5 compares the penetration and adaptation of fissure sealants in group I and group II. The mean percentage of sealant penetration in group I and group II were 97.46 and 98.03 respectively. While the mean adaptation was 0.177±0.40 for group I and 0.586±0.65 for group II.T test revealed that the difference of sealant penetrationwas not statistically significant difference between both groups (**T** = 0.380, **p**=0.705). Additionally, T test revealed a non-statistically significant difference between group I and group II regarding sealant adaptation (**T** =2.863, **P**=0.408).

Table 5: Comparison of penetration and adaptation between group I and group II

	group I	group II	T test	P
	Mean (SD)	Mean (SD)		
Penetration	97.46%±6.71	98.03%±4.47	0.38	0.705
Adaptation	0.177±0.408	0.586±0.654	2.863	0.408

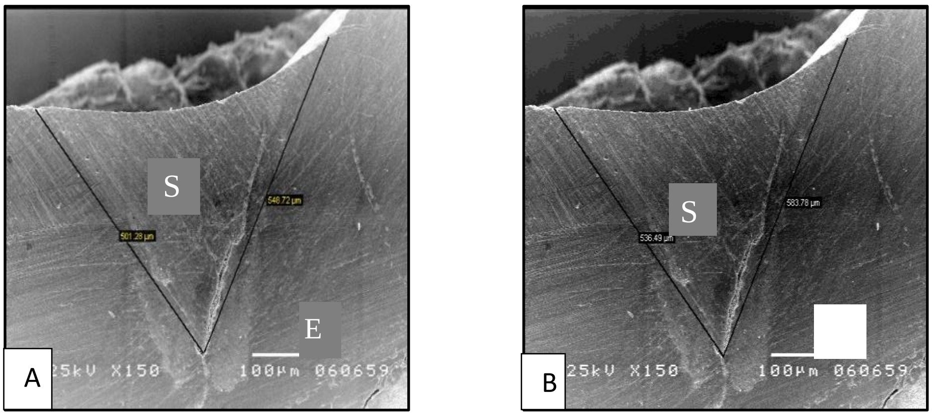

Figure 4: Scanning Electron Microscope for group I specimen showing **A**, B**:** incomplete penetration of amorphous calcium phosphate containing sealant(X150).

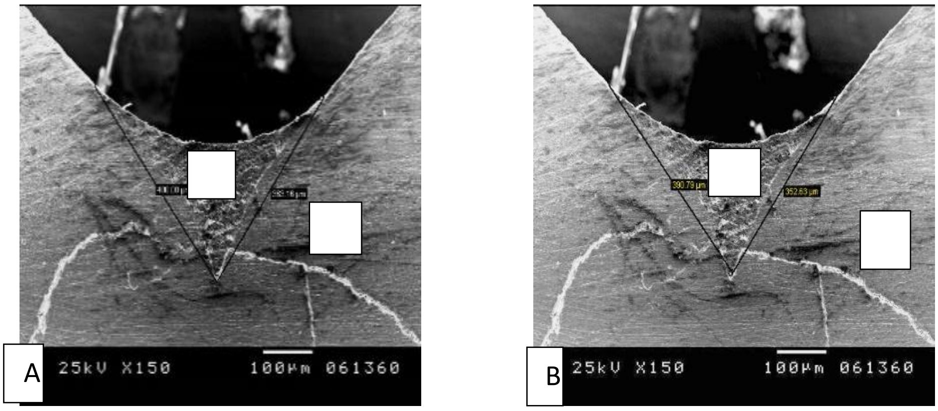

Figure 5: Scanning Electron Microscope for group II specimen showing **A**, B**:** Incomplete penetration of resin-based fissure sealant(X150)**.**

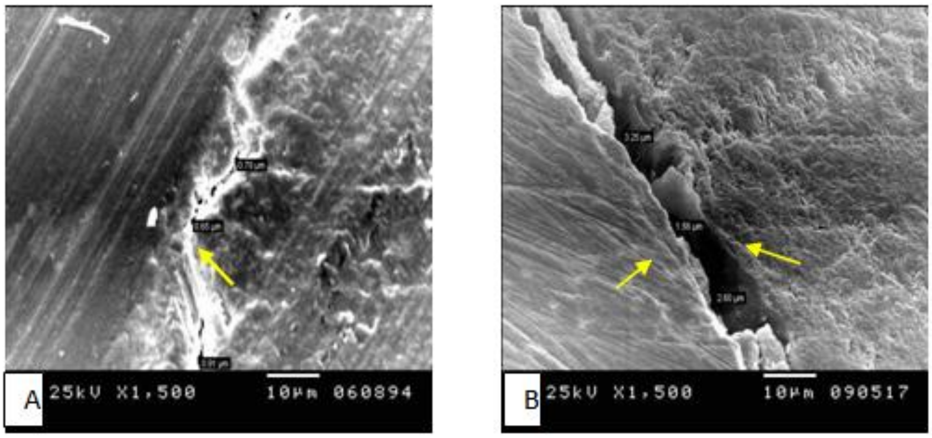

Figure 6: Scanning electron microscope for group I showing **A:** Gab present between fissure sealant material and lateral wall of tooth surface. (X1500). **B:** Gab present between fissure sealant material and lateral wall of tooth. (X1500).

IV. Discussion

Pit and fissure sealant is the most effective method in preventing occlusal caries [33]. Nowadays, resin-based sealant is the material of choice because it is cost-effective and has the ability to penetrate deep into pits and fissures. However, newly smart materials have been conducted to act as primary prevention of deep pits and fissures and stimulating the repair of tooth structure [2, 26].

Amorphous calcium phosphate has the ability to be converted into hydroxyapatite crystal which is the final stable product in the precipitation of calcium and phosphate ions [34-36]. Moreover, it is considered one of the active smart material as it only releases calcium and phosphate ions when the surrounding pH falls below5.5 and ceases when pH rises above 5.5. Thus, it has a long-lasting bioactive effect [26, 37].

In the current study, group I amorphous calcium phosphate containing sealant showed a high retention rate of 90% and 85.71% at 3 and 6 months follow up periods respectively. The present finding was higher than that reported by ***Kishor et al., 2013*** who found a retention rate of 86 % and 76% at the same follow up periods [26]. This difference in results could be attributed to different assessment criteria and diverse evaluation methods.

On the other hand, ***Ünal et al., 2015*** revealed a higher retention rate of 100% of amorphous calcium phosphate containing pit and fissures sealant at 3 and 6 months compared to the present study [23]. This results contradiction may be due to using permanent molars in the current work in contrary to primary molars used in the previous study.

Also, ***Khatri et al., 2019*** showed a lower retention rate of ACP containing sealant 81,2% and 78,1% at 3 and 6 months respectively than the current work at the same follow up periods [37]. This difference could be related to different methods of cleaning before acid etching, and different criteria for evaluation.

In the present study, group II resin-based sealant showed a retention rate of 78.57% at the end of the 9 months follow up period. This result is in agreement with ***Dukić and Glavina ,2006*** who reported a complete retention rate of 76.9% during the same follow up period[38].

Also, the current work is in a complete agreement with ***Dukiet al.,2007*** who compared resin-based sealant to other five sealant material. They found that 75.9% of teeth sealed by resin-based sealant were completely retained at the end of the study that nearly approximated the results of thisstudy [39].

However, the current study revealed a higher retention rate of resin-based sealant (78.57%) than the study done by ***Duki and Glavina 2007*** who found that the retention rate of resin-based sealant was 66.7% at the end of their study. This result disagreement could be due to the different children age used, operator diversity and longer follow up periods compared to this study [40].

Additionally, in the present study, group II resin-based sealant showed a lower retention rate than that reported by ***Schlueter et al., 2013*** after 9 months follow up period. This diversity in results may be due to different criteria of teeth selection and different age groups used. Nevertheless, results of ***Schlueter et al., 2013*** come in line with this study regarding caries prevention [41].

Also, resin-based sealant, in the current work, displayed a lower retention rate of 86.66%, 83.33% and 78.57% respectively at 1,3 and 6 months in comparison to ***Ünal et al., 2015*** who reported a retention rate of 100% at the same follow up periods [23]. This results contradiction may be related to using primary molar in the previous work compared to permanent molars used in the present study.

Furthermore, ***Kobayashi et al., 2015*** showed that resin-based sealant had retention of 83.1% at 6 months follow up period using modified Ryge Snyder criteria. This result is not in a line with the present study in which 78.57% of the resin-based sealants were completely present at the same follow up period. This difference in results may be attributed to different evaluating criteria [34].

Regarding caries prevention, the present study demonstrated that there was non-significant difference in caries prevention of ACP containing sealant and resin-based sealant ($P>0.05$) at the end of 9 months. This finding was in accordance with ***Ünal et al., 2015***despite different teeth sealed [23].

Additionally, the equivalent caries prevention of ACP containing sealant and resin-based sealant in the present work at 3 and 6 months are in line with ***Khatri et al., 2019*** although both studies use different evaluation criteria [37] .

The ability of ACP containing sealant to decrease dental caries has been attributed to release of Ca and PO_4 at pH 5.5 with subsequent remineralization of demineralized areas [25]. This might explain why carious lesion with resin-based sealant start at 3 months, while dental caries appeared at 6 months follows up periods in ACP containing sealant.

Up till now, there are no in vitro studies comparing the penetration and adaptation abilities of amorphous calcium phosphate containing pit and fissures sealant to resin based sealant

Scanning electron microscope, used in the current work, is the method of choice for evaluating both penetration and adaptation of sealant material. This may be attributed to the high-resolution image of scanning electron microscope which increased by using gold coating. Also, it reveals more details as the depth field increases yielding a characteristic three-dimensional image [42-44].

Adaptation of sealant material is considered as a determinant for caries prevention because the intimate adaptation prevents marginal gab that can induce or enhance bacterial growth in the interface that results in enamel demineralization [45].

On the other aspect, resin penetration is important factor for sealant retention which is enhanced by acid etching that strengthen the micromechanical bonding with tooth structure [43, 45].

Several authors used upper premolars for examination pit and fissure sealant under scanning electron microscope because the upper premolars are characterized by deep pits and fissures [46-49].

Selecman et al., 2007 showed that ACP containing sealant exhibited a lower microleakage when compared to the different sealant materials [10]. This result supported the current work in which Aegis group showed remarkable complete adaptation under scanning electron microscope.

However, ***Yeonheeand Hyunkyung, 2012 reported*** that Aegis showed higher microleakage when compared to 3 kinds of sealant materials [50]. This contradiction in results may be explained by different evaluation methods as the previous study used a Stereo microscope which has lower resolution image than SEM used in the current study.

The electron microscopic results of the current study revealed that resin-based sealant had intimate adaptation to the lateral wall of the tooth surface. This result is in agreement with ***Robert L et al 1990*** who reported that there was close adaptation of resin based sealant to enamel surface under SEM as well as no microleakage recorded which it is an indicator for adaptation.[51] .

However, ***Kantovitzet al., 2008*** evaluated the marginal adaptability of resin based sealant versus other sealant materials. They showed a lower adaptation of resin sealant compared to other sealant materials. This result is not in a line with the current study which could be attributed to different methods for evaluation criteria and different thermocycling preparation times [52].

On the other hand, ***Selecman et al., 2007*** reported that ACP fissure sealant had a low penetration value which is not in line with the current study. This difference may be due to use a binocular light microscope and Microscopic photograph. which is less effective than SEM used in the current study [10].

Nevertheless, ***Marks et al., 2009*** compared the penetrability and microleakage of ACP fissure sealant with acid etch preparation versus various adhesive systems. They concluded that application of sealants using phosphoric acid as a conditioning agent revealed superior results, while the use of adhesives were found to be unnecessary[53] So, this come in line with the current work which revealed a high penetration of Agis sealant material into deep pits and fissures with percentage mean 97.46.

The higher penetration ability of resin-based sealant noted in this study (Mean = 98.03 %) contradicted the findings of ***Robert L et al., 1990*** who evaluated microscopically the penetration of resin sealant into the occlusal fissures of molar teeth [51]. This could be attributed to different assessment methods because Stereo binocular microscope with descriptive evaluation criteria represented in Good, Fair or Poor was adopted in the previous work.

However, ***Prabhakar et al., 2011*** revealed that resin-based fissure sealant had long resin tag by using scanning electron microscope. These findings support the current study in which Helioseal has high penetrating ability into deep pits and fissure as revealed by SEM [43].

According to the result of this study, the non-significant difference between ACP and resin-based sealants revealed by the qualitative clinical evaluation was confirmed by the quantitative scanning electron microscope results.

V. Conclusion

Based on this study's results, the following conclusions can be obtained:

There was no significant difference between ACP containing sealant and resin-based sealants regarding to retention and caries prevention. While both ACP containing sealant and conventional resin-based sealant had a remarkable adaptation to the lateral wall of the tooth structure with a non-significant difference. Moreover, ACP containing sealant and conventional resin-based sealant had a high ability to penetrate into deep pits and fissures with a non-significant difference between both sealant materials.

VI. Recommendation

1. Amorphous calcium phosphate containing sealant is recommended as a primary caries preventive measure for newly erupted teeth with deep pits and fissure.
2. Amorphous calcium phosphate containing sealant is recommended for decreasing caries incidence in young permanent molars due to its remineralization ability and high penetration and adaptation capability.
3. Further clinical studies with longer follow up periods are required to demonstrate the long-term remineralization potential of amorphous calcium phosphate containing sealant.

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