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Effectiveness of Pit and Fissure Sealants Containing Amorphous Calcium Phosphate (ACP) and Fluoridein the Prevention of Demineralization of Adjacent Enamel: In-Vitro Study

Research Article

Ahmad Al Jawish[1], Shady Azzawy[2], Zuhair Al-Nerabieah[3*]

[1] Pediatric Dentistry Department, Faculty of Dentistry, Damascus University, Syria.
[2] Lecturer, Pediatric Dentistry Department, Faculty of Dentistry, Damascus University, Syria.
[3] Pediatric Dentistry Department, Faculty of Dentistry, Damascus University, Syria.

Abstract

Aim: The aim of this study was to evaluate the effectiveness of amorphous calcium phosphate and fluoride-containing pit and fissure sealantsin the prevention ofdemineralized of adjacent enamel compared with conventional sealants.
Materials and Methods: The sample consisted of 45 buccal and lingual slabs of sound third molars free of cracks, or stains and demineralized areas. The specimens were randomly assigned into three groups; 15 in each. Box-shaped cavities (4 x 2 x 1 mm) were prepared on the buccal or lingual surfaces for the application of study's materials withinthe cavity in each slab. In the first group; Amorphous Calcium Phosphate (ACP)-containing sealant Aegis®, andin the second group fluoride-containing sealant(Clinpro™) was applied,while the third group conventional sealants without fluoride(Helioseal® Clear) was applied. After the application of the material, two coats of acid-resistant nail varnish were applied to the tooth surface, leaving a window not less than approximately 1 mm wide surrounding the occlusal margin of each cavity.Then the specimens were immersed in demineralization and re-mineralization solutions.Microhardness measurements were carried out at the exposed demineralized enamel layer adjacent to the sealants, and also the sound area of enamel that is not exposed to demineralization.
Results: Statistical results showed that there were no statistically significant differences between (ACP) and fluoride containingpit and fissure sealants, with regard to the amount and the percentage changes in the microhardness between the sound and the demineralized part of the tooth, while there were significant differences between pit and fissure (ACP) and fluoride containing in comparison with the conventional sealant.
Conclusion: The amorphous calcium phosphate and fluoride-containing pit and fissure sealants have some potential effect in the prevention of demineralization of adjacent enamel.

Keywords: Pit and Fissure Sealants; Amorphous Calcium Phosphate; Fluoride; Microhardness; Demineralization.

Introduction

Dental sealants are considered one of the best available prevention manner forpit and fissure caries. Sealants also can prevent the development of dental caries before it reaches the end-stage process which called cavitation's. Basically, the aim of pit and fissure sealants is preventing the development process of caries, which is achieved by sealing the surface, and preventing bacteria from attaching to enamel surface [1].

Micro leakage and secondary caries between the sealant and tooth surface are among the biggest issues with these materials. Several studies have been conducted on sealant materials andapplication methods to improve their properties like retention and marginal integrity which provide pit and fissure sealant protection efficacy. Since the retention of the sealant is not permanent, this physical effect could be enhanced with the ability of releasing fluoride ions [2].

A recent enhancement of sealants is the introduction of amorphous calcium phosphate (ACP)-containing pit and fissure sealants to take advantages of the expected use of calcium and phosphate ions in enamel remineralization and caries prevention [3, 4].

***Corresponding Author**:
Zuhair Al-Nerabieah,
Pediatric Dentistry Department, Faculty of Dentistry, Damascus University, Al-Mazzeh St., Damascus, P.O Box 30621, Syria.
Tel: +963969960118
E-mail: Zuhairmajid@gmail.com

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(ACP) has preventive and restorative properties that justify its use indental cements, sealants, composites and orthodontic adhesives. Studies have reported that ACP-filled composite resins have shown the ability to repair 71% of the lost mineral content of decalcified enamel [5].

During dental caries, when the pH value of oral environment is reduced below the value of (5.8), Hydroxyapatite (HAP) is filtered from the enamel surfaces [6, 7]. In these conditions, ACP can induce the formation of HAP, which in turn helps the dental enamel in remineralization [8].

Fluoride-containing pit and fissure sealants have characteristics that are not present in conventional non-fluoride sealants, in terms of their stability and survival. In additionto being considered as a reservoir of Fluoride ions and thus enhance the formation of fluoroapatite in the dental enamel [9].

The aim of this study was to investigate the ability of amorphous calcium phosphate (ACP) comparing with fluoride containing pit and fissure sealants in the prevention of demineralization of adjacent enamel using a surface microhardness taste.

Recently some studies have been indicated that both amorphous calcium phosphate and fluoride-containing sealants have a role in protecting the adjacent enamel against demineralization. However, there are limited studies on the potential remineralization efficacy of ACP-containing sealants on the adjacent enamel [3, 4, 10].

Materials And Methods

Ethical approval was obtained from the Damascus University Research Scientific Committee. CRIS guidelines for reporting In-vitro studies was applied in this study. This study was carried out on fresh lyextractednon carious third molars. The roots were removed perpendicular to long axis of the tooth by a diamond bur with air water coolant to prevent enamel damage. It was then examined by an optical microscope under magnification x 20 and also by a DIAGNOdent device to detect any caries or demineralized areas or cracks on the labial or lingual/palatal surfaces.

The buccal and lingual surfaces were cleaned by a nylon brush on a low- speed handpiece in order to remove the fluoride rich surface enamel layer and to obtain a relatively flat surface, the buccal and lingual aspects of each tooth were polished with a series of aluminum oxide disks. Then the molar segments were separated into two buccal and lingual by a separatordisc. Highly convex sections were excluded due to the difficulty of applying the microhardness taste procedures. So that 45 sound specimens, free of caries, cracks, pigmentation and demineralization were selected.

Then, using high speed handpiece with sufficient cooling irrigation, a window waspreparedin the middle and cervical thirds of the samples with dimensions of 2 mm wide, 4 mm long and 1 mm deep by aninvertd conetungsten carbide bur (A 208) where the dimensions were determined by a millimeter ruler and the depth was determined by entering only with a 1 mm capillary head.

The 45 samples were numbered and randomly divided into three groups, where they were preserved in plastic bottles with distilled water until the experiment was initiated. The studied 3 sealant materials were filled within the prepared window according to the manufacturer's instructions for each material, in a role of 15 window cavity for each.

Study Groups

The first group: consisted of 15 windowin which the amorphous calcium phosphate (ACP)-containing sealant was filled (Aegis® - opaque white; Bosworth Co. Ltd.).

The second group: consisted of 15windowin which the fluoride-containing sealant was filled (Clinpro Sealant ™, 3M ESPE).

The third group: consisted of 15 windowin which the conventional sealant was filled (Helioseal® Clear – Evoclar Vivadent).

Then the surface of each section was isolated by two coats of acid resistant of nail varnish except 1 mm from the side of the occlusal part of the sealant. The demineralization phase of the samples was then carried out by placing samples in alternating solutions of demineralization and re-mineralization.

Demineralization Solution consisted of 2 mmol of chlorine calcium, 2 mmol of monosodium phosphate and 50 mmol of acetic acid. Then, the solution pH was modified by adding sodium hydroxide at a concentration of 0.1 so that we obtained a solution of pH equal to 4.5 [11]. While Re-mineralization Solution consisted of 2 mmol of calcium chlorine and 2 mmol of monosodium phosphate. Then, the solution pH was modified by adding sodium hydroxide at a concentration of 0.1 so that we obtained a solution of pH equal to 6.8 [11].

All samples were immersed in the first demineralization solution for four hours, then each sample was washed well with 5 ml of distilled water and then immersed in the second remineralization solution for 20 hours the same procedure was repeated for 4 days [3].

Microhardness Measurements

After the end of demineralization and remineralization cycling, in allstudied surfaces the nail varnish layer was removed, where a clear demineralization lesion appeared in the surrounding not isolatedenamel surface. The microhardness test was then carried out by GALILEO MICROSCAN OD, (Italy) based on the Vickers (VHN) unit at the Faculty of Mechanical Engineering-Damascus University, in both the demineralized area of the enamel as well as the sound area of the enamel for the same sample which was protected by nail polish. Three measurements on different locations were made in each area and the mean of these measurements was takenby applying 100 gm of force for 15 seconds.

Surface microhardness (HV) is calculated by the following equation:

$$HV= (1854 \times P)/D^2$$

P:applied loadD:the average diameter of the impact left by the pyramid, measured in microns. D= (d1+d2)/2 micrometre
P=100 grams

Statistical Analysis

The micro-hardness was measured at two different measurement sites (in theDemineralized part and the sound part of the tooth surface) for each dental section.

Data was analyzed using statistical software (IBM SPSS Statistics version 22).

The amount and the percentage change in the microhardness between the demineralized and the sound part of the tooth were calculated for each of the dental sections.

One-way analysis of variance (ANOVA) test was performed to study the significance of differences in mean surface microhardness values between the study groups at a significance level of (0.05). The post-hok Bonferroni test was performed to study the significance of bilateral differences in the mean surface microhardness values in the demineralized part of the tooth between study groups.

T-student test was performed to study the significance of the differences between the mean surface microhardness in the demineralized and the sound part of the tooth according to the sealants used in the study sample.

Results

This in vitro comparative study was conducted to investigate the ability of both amorphous calcium phosphate and fluoride in the prevention of demineralization of the adjacent enamel and compare them with conventional sealant using surface microhardness test.

The study sample consisted of 45 dental sections taken from 25 healthy third permanent molars, the sections that met the criteria for entry in the research sample were randomly divided into three groups according to the material used;containing amorphous calcium phosphate (ACP), containing fluoride Clinpro Sealant; or conventional Helioseal.

The meanvalues of microhardness in the sound part of the tooth were (3.52.95-345.96-391.26) in the three groups (ACP, fluoride,conventional sealant).

The highest mean value of the surface microhardness in the demineralization part of the sample was in the (ACP) group as it was (204.81), while the lowest mean value was in the conventional group (124.08), while in fluoride group was (161.92) (Table 2).

According to the results of the one-way analysis of variance (ANOVA), there were no statistically significant differences between surface microhardness means of the three groups in the sound part ($p>0.05$),Whilethere were statistically significant difference between surface microhardness means of the three groups in the demineralized part ($p<0.05$),(Table 3).

According to the results of the (Bonferroni test), which was conducted to study the significance of the bilateral differences in the amount of change in the microhardness between the demineralized and the sound part of the tooth between the study groups. Statistically significant differences were found between each of the (ACP) and (Helioseal) groups, as there was a difference in the (Clinpro) and (Helioseal) groups ($p<0.05$). While there were no statistical differences between the group of (ACP) and (Clinpro) ($P> 0.05$).(Table 4).

Discussion

Deep Pits and fissures in posterior teeth are the most prone sites forthe accumulationof plaque and food debris. Althoughsealants can reduce the incidence of developingcariouslesions, Secondary carries may occur as a result of bacteria invasion due to microleakage [12].

Therefore, there is a need for materials with a substantial capacity

Table 1. Shows the material used in the study.

Group	Manufacturer	Main components	sealant	Description
1	Bosworth®-	UDMA, mono-anddi methacrylate resins	Aegis opaque white	Amorphous Calcium Phosphate-containing resin-based sealant
	Company (USA)	TLV-TWA: 15 mg/m³ TWA for ACP N/A for resin		
2	3M ESPE,St. Paul, MN, USA	Bis-GMA, TEGDMA, EDMAB, CPQ, TiO2, amorphoussilica, Tetrabutylammoniumtetrafluoroborate	Clinpro Sealant	Fluoride-containing resin-based sealant
3	İvoclarVivadent Ets.,(Schaan,	Bis-GMA, TEGDMA (>99wt %)	HelioSeal clear	Conventional resin – based sealant
	Liechtenstein)	Additional contentsare stabilizers andcatalysts (<1 wt. %)		
Abbreviations	BisGMA=bisphenolaglycidylmethacrylate; N/ A = Not available; TEGDMA=triethyleneglycoldimethacrylate; TLV-TWA = Threshold Limit Value-Time-Weighted Average; UDMA =urethane dimethacrylate.			

Table 2. Shows the mean, standard deviation, standard error, minimum and maximum hardness HV in the study sample according to the sealants used and the measurement area.

Measurement area	Sealant Groups	n	Mean	Standard deviation	Standard error	Min	Max
Demineralized area	Bosworth Aegis	15	204.81	40.73	10.52	126.8	260.5
	Clinpro Sealant	15	161.92	57.66	14.89	68.73	320.65
	Helioseal	15	124.08	51.34	13.26	64.9	216.3
Sound area	Bosworth Aegis	15	352.95	39.72	10.26	256.8	440.4
	Clinpro Sealant	15	345.96	58.31	15.06	254.6	436
	Helioseal	15	391.26	70.43	18.18	306.5	550.4

Table 3. Shows the results of one way analysis of variance (ANOVA) to study the significance of differences in the average amount of microhardness between the three groups studied material sealants used in the study sample according to the location of measurement.

Variable studied = the amount of microhardness			
The location of measurement	**F value**	**P value**	**Indication of differences**
demineralized area	9.637	0	There are significant differences
Sound area	2.693	0.079	There are no significant differences

Table 4. Shows the results of the bilateral comparison in the Bonferroni method to study the significance of the bilateral differences in the amount of change in the microhardness between the demineralized and the sound part of the tooth between the study groups of sealants used in the study sample.

Variable studied = the amount of change in the microhardness between the demineralized and the sound part					
Sealant I	**Sealant J**	**(I-J)**	**Standard error for the difference**	**P value**	**Indication of differences**
ACP	Clinpro Sealant	35.91	19.96	0.238	There are no significant differences
	Helioseal	119.96	19.96	0	There are significant differences
Clinpro Sealant	Helioseal	83.14	19.96	0	There are significant differences

for remineralization. On this basis, the occurrence of secondary caries as a result of microleakage can decrease. Bioactive substances, which increase the concentrations of calcium and phosphate or release fluoride within the lesion to levels higher than those in surrounding oral fluids, have the potential to promote remineralization [2].

In this present study, the remineralization potential of amorphous calcium phosphate and fluoride-containing pit and fissure sealants was evaluated compared to conventional sealants using the Vickers hardness.

The meanof microhardness surface was in the sound part of the sample, as it reached (345.96-352.95-391.26) in each of the study groups which indicates the homogeneity of the sample in the sound part.

This is consistent with the results of the study of Zawaideh et al, where in their study there were no statistically significant differences in the mean values of microhardness surface of the three groups before demineralization, which corresponds to the sound part of the sample in our research [3].

There was a statistically significant difference between the study groups in terms of surface microhardness in the demineralized part, ($p<0.05$).It can be noted form table (2) that the highest value of microhardnesswas in (Aegis) group.

In the Zawaideh et al study, the largest value ofsurface microhardness, was also in the Aegis group.And concluded that the ACP and fluoride - containing pit and fissure sealant have the potential to inhibit enamel demineralization of adjacent enamel compared with conventional sealants without fluoride [3].

Alsaffar et al [4] evaluated the effect of some types of pit and fissure sealants in protecting adjacent enamel from acid demineralization. They concluded that both amorphous calcium phosphate and fluoride containing pit and fissure sealants may provide some protection against demineralization of the adjacent enamel compared with conventional sealants [4].

The current results were also consistent with the results of the study of Silva et al., [13] who found in their study that the use sealant containing amorphous calcium phosphate (Aegis) and sealant containing fluoride (Fluor shield) promote the remineralization of artificially carious lesions on enamel surfaces [13].

The results of the present study showed that the fluoride pit and fissure sealant (Clinpro) have the possibility of prevention of demineralization, compared with the conventional sealant (Healioseal) according to this study results of the amount of change and the percentage of change.

It was found that the presence of fluoride in sealants reduces the dissolution of the enamel and stimulates the remineralization, and thus inverts the caries process at an early stage towards remineralization [14].

Fluoride release from sealants may occur as a result of the porosity of these substances, and may also be due to the non-bonding of crystals or ions of fluoride to strongly polymerized molecules, and may be due to the deposition of fluorine crystals on the surface of the resin [15].

The results of the current study is in agreement of with the results of the study of Salar et al [16], who found that the integration of fluoride with sealants increased demineralization inhibition as compared with the conventional non-fluoride based but less than those based on (GIC), This study indicates that the new fluoride-releasing sealant substantially reduces the amount of enamel demineralization adjacent to the material [16].

Choudhary et al [2] concluded that both ACP and fluoride containing sealants had the potential for remineralization.The release of amorphous calcium phosphate molecules and the formation of fluorapatite is responsible for remineralization in their study groups that included the amorphous calcium phosphate containing sealant, fluoride containing and conventional sealants. However, they applied the sealants on premolars, and used a two-week pH cycling. Specific changes in tooth surfaces, sealant and white areas around them were examined and remineralization was calculated using (SEM). The ACP group and the fluoride group showed white areas on the dental surfaces adjacent to the sealant, which is an indication of the remineralization of the adjacent areas, While these areas did not appear in the group of conventional sealants [2].

The results of the Delben [10] study showed that ACP inhibited the demineralization in the deep areas of the enamel, while fluoride products had a greater effect in the surface areas of the enamel, while the combination of ACP and F remineralization factors was more effective In the prevention of demineralization. They evaluated the discontinuity probability of caries of ACP-containing sealant using (SMG) synchrotron microtomography. They used bovine enamel blocks divided into 5 groups, using a conventional non-fluoride-based sealant, amorphous calcium phosphate based, experimental sealant containing both amorphous calcium phosphate and fluoride, and resin-modified glass ionomer cement. Theblocks were presented for a demineralizationcycling. Fluoride groups showed a positive rate of mineral gain, which was clearly visible in the outer layer of the enamel. The ACP group showed a loss of demineralization in the outer layer compared to the fluoride group, although it inhibition demineralization in the deep areas of the enamel, while the combination between (ACP and F) was highly effective in preventing demineralization [10].

The results of the current study differed with the results of Kantovitz et al [17], which aimed to assess the effect of the fluoride-containing and non-fluoride-containing sealants on the enamel mineral loss on different distances from the sealant margin, and to verify the effectiveness of these materials in the release of fluoride, as they concluded that is the fluoride-containing and non-fluoride resins sealants did not prevent the loss of minerals and they indicated the necessity of adopting additional preventive procedures. All groups were subjected to thermo and pH cycling-for 15 days they also used the Knoop microhardness scale, and evaluated the loss of minerals at different distances and depths from the sealant margin [17].

We also differed with the results of the study of Vatanatham et al [18], where they concluded that there is no significant difference between the fluoride and non-fluoride-containing sealants in the loss of minerals from ncipient enamel artificial carious lesionsinduced in premolars by means of 9-day demineralization procedures in which they used fluoride and non-fluoride sealants as they considered that the short and long-term release of fluoride from the restorative material depended on the resin base, its manufacturing technology and its fluoride content and on oral health conditions [18].

Finally, there are limitations to this study as there is a difference between the oral environment conditions and laboratory procedures, where there are several factors that can affect the durability and stability of the sealants and the reduction of demineralization the area adjacent to the sealants and thus a change in the amount of microhardness (such as the type of acid attacks and their frequency and temperature the body, the nature of saliva, oral health and nutritional habits, and the difference between the conditions of clinical and laboratory application of the substance).

Conclusions

The amorphous calcium phosphate and fluoride containing pit and fissure sealants can provide an added advantage over conventional sealant in protecting the adjacent enamel layer and protecting it from demineralization.

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