Assessment and Comparison of Nanoleakage and Resin Tag Length of Three Different Pit and Fissure Sealants: An *In-vitro* Scanning Electron Microscope Study.

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Abstract: Aim: To assess nanoleakage and resin tag length of three different Pit and Fissure Sealants using scanning electron microscope. **Material and Methods:** The occlusal surfaces of 15 intact extracted human maxillary first premolars (divided into three equal groups), were cleaned with pumice, etched with 37% phosphoric acid for 15 sec, rinsed and dried. Premolars were then sealed with; Group A: Fisseal Flowable composite, Group B: Vertise Flow composite and Group C: Embrace WetBond. Teeth were stained with modified silver staining technique. With the aid of SEM, nanoleakage was measured using the Dye absorbance method and length of resin tags were determined. Data management and analysis were performed using Statistical Package for Social Sciences (SPSS) version 17. Comparisons between the different sealants were done using the Kruskal-Wallis test. The test was considered significant when $p<0.05$. **Results:** Nanoleakage is significantly less, and length of resin tags is significantly longer in Vertise Flow group followed by Embrace WetBond then Fisseal groups, which were not significantly different from each other, ($p < 0.05$). **Conclusion:** This study indicated that, there is a negative correlation between resin tag length and nanoleakage; the longer the resin tags, the lesser the nanoleakage, and the better the cariostatic action of Pit and Fissure Sealants and the use of Vertise Flow composite as good alternative for sealing pits and fissures is recommended.

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Key words: Nanoleakage, length of resin tags, Pit and Fissure sealant, Flowable composite, Vertise Flow composite, Embrace WetBond, SEM, Dye absorbane method.

1. Introduction

The major concern of modern dentistry, mainly for the last decade, has become focused on reducing patients risk for caries, stimulating preventive measures, and preserving tooth structure, indicating, as often as possible, non-invasive conservative techniques instead of proceeding with an invasive healing treatment [1,2] .

Deep pits and fissures (Deep, narrow I-shaped and K shaped) which are not accessible for cleaning, have the highest caries susceptibility and have always remained an area of concern for the dentists[3]. Sealing of these caries susceptible sites is considered an effective method of caries prevention [4]. Pit and Fissure Sealants are defined as the materials, which are placed in the pits and fissures of teeth in order to prevent or arrest the development of dental caries [5].

Several types of resin, both filled and unfilled, have been employed as Pit and Fissure Sealants. These resin systems includes Cyanoacrylates, Polyurethanes, and Bis-GMA. The main component of the Fissure Sealant is Bis-GMA resin. The success of the sealant technique is highly dependent on obtaining and maintaining an intimate adaptation of the sealant to the tooth surface [6].

The marginal sealing ability of a sealing material is extremely important for success of treatment. Weak sealing can lead to marginal leakage, resulting in bacterial invasion, caries initiation and progression underneath the restoration. In vitro microleakage studies can predict the marginal integrity of restorative materials [7].

Microleakage may support the caries process beneath the sealant; hence the ability of the sealant to adequately seal the pits and fissures and prevent microleakage is essential. Microleakage assessment may be qualitative or quantitative with different systems, including both simple and computer based methods. Dye penetration has been used in several studies, to assess the presence of marginal leakage at the sealant/enamel interface [8].

A sealant is effective in preventing caries, only when it is successfully retained in the fissures. Hence the retention becomes a major factor, influencing the efficacy of the sealant. The retention of resin based Pit and Fissure Sealant is through micromechanical interlocking between the resin and the etched enamel. Mechanical retention of sealant is the direct result of resin penetration into the porous enamel forming tags. This occurs by capillary action.

The resin monomer polymerizes and becomes interlocked with the enamel surface [9].

Thus, it can be postulated that, to a certain extent the longer the resin tags formed, the lesser will be the microleakage. Other than surface tension, the viscosity of the sealant also influences the penetration of sealants [10]. With low viscosity sealants, there is a greater potential of the sealant to flow, spread and penetrate more rapidly etched enamel surface [11].

A Fissure Sealant flowable composite resin showed good results [12]. The applicability of Flowable restorative systems in dentistry has increased, mainly because of their beneficial properties which include low viscosity, low modulus of elasticity and easy handling. Flowable composite materials have better abrasion resistance and, thus, provide a better retention than a conventional unfilled resin [13-15].

However, clinicians have faced some challenges associated with effectively placing traditional Pit and Fissure Sealants; these challenges include moisture control, manipulative technique sensitivity and time consuming procedure. High quality polymeric restorative materials are desirable specially when treating children with unpredictable tolerance, patience and cooperation [16].

Taking into account all of the previous, dental manufacturers concentrated their efforts to offer a bonding procedure that involves less application time, and can be achieved with less steps, less technique sensitive; thus increasing their potential success.

One of these materials is the Self-Adhering, flowable resin -based Pit and Fissure Sealant (Vertise Flow) which is the first self-adhering resin composite. It is the logical continuum in the chain of product development aiming towards simplification and ease of application of dental composites. The bonding mechanism with tooth structure is a chemical bonding achieved via the GPDM (Glycerophosphate dimethacrylate) between phosphate functional groups of GPDM monomers and the calcium ions of enamel and dentine (Optibond Technology) [17].

By including the bonding in its formulation, Vertise Flow eliminates the additional steps of etching/priming/bonding, otherwise necessary to bond a resin composite to dentin and enamel [18].

Vertise Flow composite offers high bond strength, high mechanical strength, and other physical attributes comparable to traditional Flowable composites. One of the applications of Vertise Flow is its use as Pit and Fissure Sealant [19-20].

Another material is the Moisture-Tolerant, resin-based Pit and Fissure Sealant (Embrace WetBond) which is the first sealant that could effectively tolerate some degree of moisture when applied to the etched enamel surface without compromising the bond and without requiring a separate bonding agent [21,22].

Embrace WetBond has proven its ability to effectively seal teeth at risk for carious lesions. Clinicians experience the effectiveness of the material at placement through its seamless integration with tooth structure. The improved adhesion of Embrace WetBond along with its ability to create smooth margins, contribute to its longevity and clinical effectiveness [23]. It does not include Bis-GMA and therefore contains no Bisphenol-A, which has generated some public concern regarding potential harmful effects [24].

However, there appears to be few research studies comparing such materials with that of conventional sealants. Therefore, the purpose of this study is to evaluate nanoleakage and length of resin tags of Flowable composite resin (Fisseal), Self-Adhering (Vertise Flow) and Moisture-Tolerant (Embrace WetBond) Pit and Fissure sealants. Each has a claimed advantage.

2. Materials and Methods

Fifteen sound freshly extracted maxillary first premolars of patients indicated for orthodontic treatment were collected at Pedodontic and Dental Public Health Department, Faculty of Oral and Dental Medicine, Cairo University.

The collected teeth were cleaned of any adherent deposits debris with an ultrasonic scaler (EMS, Switzerland) and were stored in deionized water [25] for a maximum of one month at room temperature.

Pretreatment of the occlusal surfaces was done by cleaning the teeth with pumice slurry, washed and dried.

The teeth were then randomly divided into three equal groups (5 premolars each):

Group A: Sealed with Flowable composite resin Pit and Fissure Sealant (Fisseal).

Group B: Sealed with Self-Adhering, flowable resin- based composite (Vertise Flow).

Group C: Sealed with Moisture-Tolerant, resin-Based Pit and Fissure Sealant (Embrace WetBond).

Procedure for Pit and Fissure Sealant application:

Group A (Fisseal):

According to the manufacturer's instructions occlusal surfaces of the teeth were etched with 37% phosphoric acid (Technical and general ltd, London, England) for 15 sec and thoroughly rinsed with water. The teeth were then dried with a mild oil-free air stream to achieve a characteristic frosted chalky white appearance of enamel.

Fisseal was dispensed onto the prepared occlusal surfaces using a dispensing tip. Polymerized for 30 sec using light curing unit (LED: Bluephase ® C5 curing-light, 500 mW/cm2, 430-490 nm, Ivoclar Vivadent AG FL-9494 Schaan/Liechtenstein, Austria).

Table (1): The following is the list of the Pit and Fissure Sealants used in the study.

Pit & Fissure Sealant	Composition	Manufacterur	Lot #
Fisseal	BISGMA, diurethane dimethacrylate, BHT, benzotriazol derivative and NaF	Promedica, Germany	Art. No.2472
Vertise Flow	Hydroxy Ethyl MethAcrylate (HEMA), 4 Methoxyphenol (MEHQ), Zinc Oxide, pigments. 70% filler loading: nano-ytterbium fluoride, pre-polymerised fillers and nanoparticles.	Kerr Corporation California, U.S.A.	Part #34401 lot 3457087
Embrace WetBond	Glass Filled Resin 36.6 % ,di-, tri- and multifunctional acrylate monomers into an advanced acid-integrating chemistry	Pulpdent Corporation Watertown NA USA	02471-0780

Group B (Vertise Flow):

Occlusal surfaces of the teeth were etched, rinsed and dried as previously mentioned. According to the manufacturer's instructions Vertise Flow was dispensed onto the preparation using dispensing tip. A thin layer of Vertise Flow was brushed onto the occlusal surface with moderate pressure for about 15 sec. Excess material around the margins was removed using the provided brush, and then light cured for 20 sec.

Group C (Embrace WetBond):

Occlusal surfaces were etched and rinsed with water, as before. With Embrace WetBond, the typical dull, frosted appearance of the etched surface is not desired. Rather, the surface was lightly dried (very slightly moist with a glossy appearance). To accomplish this, a cotton pellet was used to remove the excess moisture. The sealant Embrace WetBond was applied as per manufacturer's instructions, followed by light curing for 40 sec. All the treated teeth were stored in deionized distilled water.

Staining of Teeth:

The root apices of the treated teeth of each group were covered with sticky wax (Dentsply DeTrey, Bois Colombes, France), while their entire surfaces were coated with two layers of nail varnish except for the sealed occlusal surfaces.

A modified silver staining technique *(Tay et al., 2002)* [26] was used with basic 50wt% ammoniacal silver nitrate (pH=9.5). The solution was freshly prepared by dissolving 25 g of silver nitrate crystals in 25 ml of distilled water.

Concentrated (28%) ammonium hydroxide was used to titrate the black solution until it became clear as ammonium ions complexes the silver into diamine silver ($[Ag(NH_3)_2]^+$) ions. This solution was diluted to 50 ml with distilled water to achieve a 50wt% solution. The teeth were immediately immersed into the freshly-prepared ammoniacal silver nitrate solution in total darkness for 24 hrs, followed by thorough rinsing with running distilled water for 5 min.

The stained teeth were then placed in a photo developing solution (Agfa, Belgian) for 8 h under fluorescent light to reduce the diamine silver ions into metallic silver grains within the voids along the bonded interfaces[27]. After removal from the developing solution, the teeth were placed under running distilled water for 5 min.

The stained teeth were then sectioned; their cut surfaces were finished and polished using a carbide stone. The polished sections were then decalcified using 37% phosphoric acid for 15 sec to etch away any enamel mineral component not protected by sealants and then rinsed and stored in distilled water.

Procedure of SEM for evaluating nanoleakage and length of resin tags:

The tooth sections were dried thoroughly under heat lamp, and mounted on brass rings using a non conductor tape made of carbon. This was then applied to the sections, in the areas that did not need scanning. These mountings were then placed inside an ion spluttering device (Emitech K550X sputter coater, England) for 30 min using vacuum evaporation at 200 -300 A^{o}[28].

The gold spluttered sections were then examined using SEM ; SEM Model Quanta 250 FEG (Field Emission Gun) attached with EDX Unit (Energy Dispersive X-ray Analyses), with accelerating voltage 30 K.V., magnification 14x up to 1000000 and resolution for Gun 1n (FEI company, Netherlands) and photographs of the sections were obtained. The measurements of silver penetration were calculated directly on the SEM monitor, using a multi-point measuring device, in steps of approximately 100 µm [29].The nanoleakage values were expressed as the bands showing silver deposition, Figs. (1-3). The resin tag lengths were then measured, Figs. (3-5) and the average of each photograph was calculated.

Statistical analysis

Data management and analysis were performed using Statistical Package for Social Sciences (SPSS) version 17. Comparisons between the different sealants were done using the Kruskal-Wallis test, a nonparametric test equivalent to the one way analysis of variance, to use in non-normally distributed variables. All p-values are two-sided. The test was considered significant when $p<0.05$.

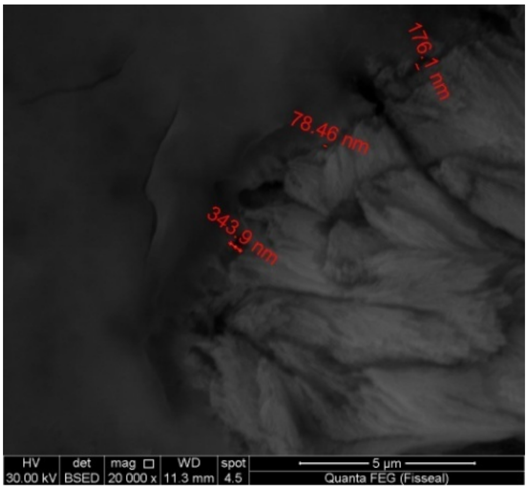

Fig (1): SEM image showing nanoleakage values (Fisseal)

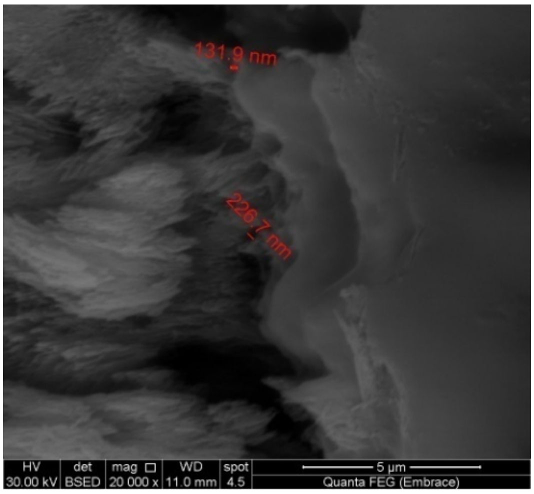

Fig (2): SEM image showing nanoleakage values (Vertise Flow)

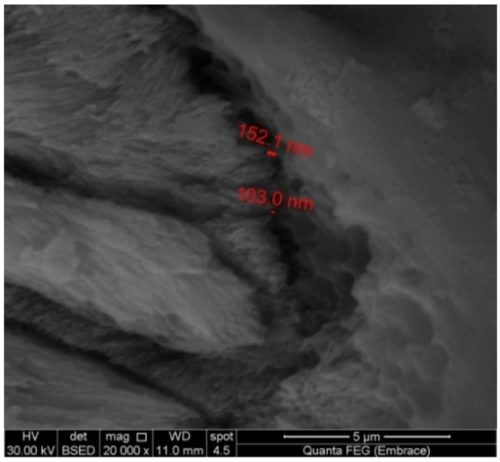

Fig (3): SEM image showing nanoleakage values (Embrace WetBond)

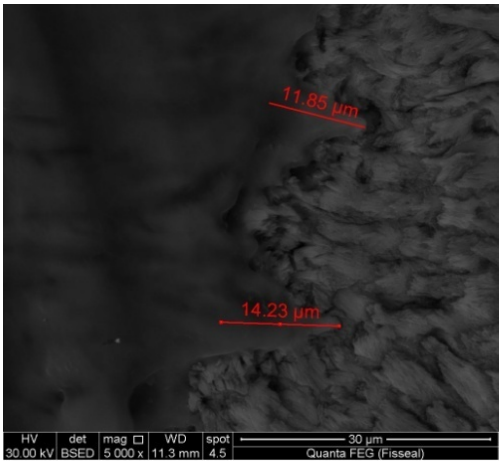

Fig (4): SEM image showing the length of resin tags (Fisseal)

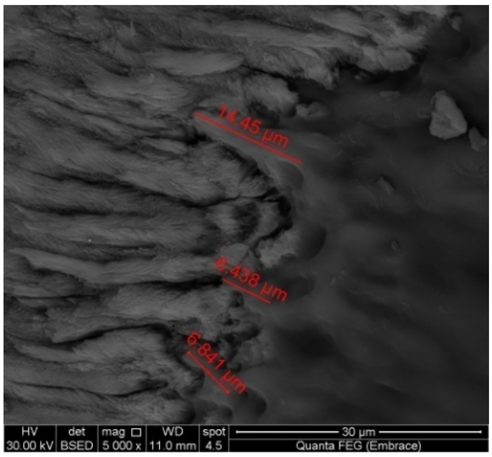

Fig (5): SEM image showing the length of resin tags (Vertise Flow)

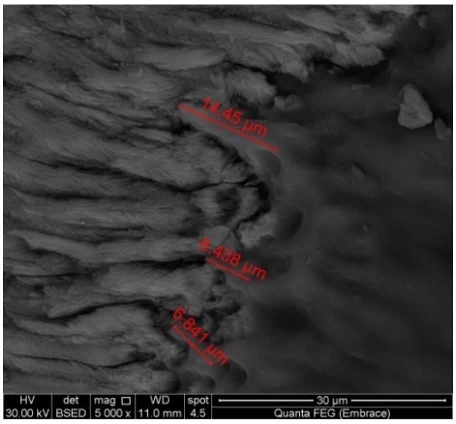

Fig (6): SEM image showing the length of resin tags (Embrace WetBond)

3. Results:

The purpose of this study was to evaluate nanoleakage and length of resin tags of Fisseal Flowable composite, Vertise Flow composite and Embrace WetBond Pit and Fissure sealants.

Table (2) and Fig (7); show the mean nanoleakage values of the three groups under study. For Group A (Fisseal) the mean nanoleakage value is 10.1± 2.9nm , for Group B (Vertise Flow) the mean nanoleakage value is 4.9± 2.2nm and for Group C (Embrace WetBond) the mean nanoleakage value is7.9± 2.5nm.

Vertise Flow group showed the least nanoleakage followed by Embrace WetBond then Fisseal groups; which both are not significantly different from each other (p <0.05).

Table (2) and Fig (8); show the mean length of resin tags of the three groups under study. For Group A (Fissael) the mean length of resin tags is 153.9± 91.3um, for Group B (Vertise Flow) the mean length of resin tags is 290.7± 65.2 um and for Group C (Embrace WetBond) the mean length of resin tags is 193± 75.7um.

Length of resin tags is significantly longer in Vertise Flow group followed by Embrace WetBond then Fisseal groups; which are not significantly different from each other (p<0.05).

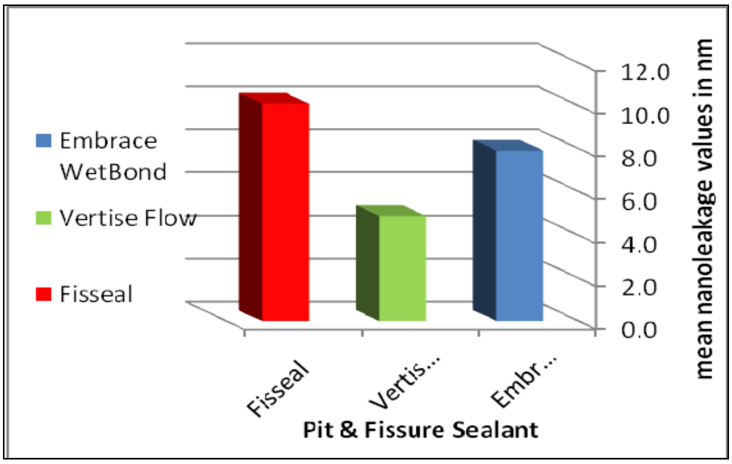

Fig (7): A bar chart showing the mean nanoleakage values of the three Pit and Fissure Sealants under study

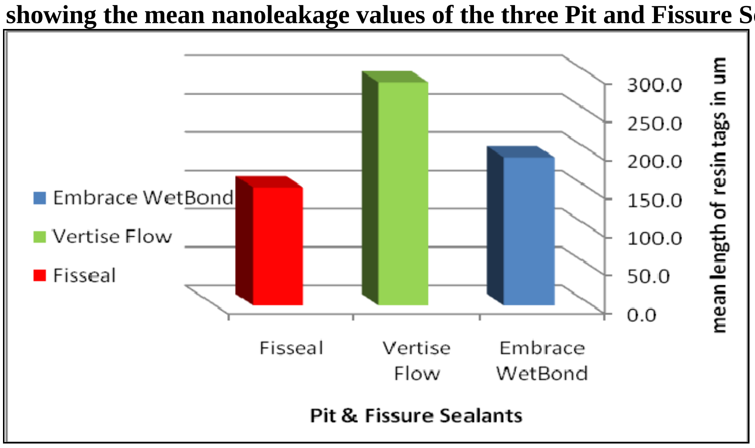

Fig (8): A bar chart showing the mean length of resin tags of the three Pit and Fissure Sealants

Table 2: The mean values of nanoleakage and length of resin tags of the three Pit and Fissure sealants under study

	Fisseal		Vertise Flow		Embrace WetBond		
	Mean	±SD	Mean	±SD	Mean	±SD	*p*-value
Nanoleakage in nm	10.1	±2.9	4.9	±2.2	7.9	±2.5	0.001
Length of resin tags in um	153.9	±91.3	290.7	±65.2	193	±75.7	0.004

4. Discussion

The occlusal surface, especially the pits and fissures of posterior teeth have been recognized for their high caries susceptibility over many years[30]. It is undoubtedly more vulnerable due to the unique morphology of the pits and fissures. Occlusal pits and fissures vary in shape, but are generally narrow and tortuous, and are considered to be an ideal site for the retention of bacterial and food remnants. This is because the morphology renders the mechanical means of debridement inaccessible [31].

The cariostatic properties of sealants are attributed to the physical obstruction of the pits and fissures. This prevents colonization of the pits and fissures with new bacteria and also prevents the supply of fermentable carbohydrates to any bacteria remaining in the pits and fissures [32].

Requisites of an efficient sealant include: A viscosity allowing penetration into deep and narrow fissures, adequate working time, rapid cure, good and prolonged adhesion to enamel, low sorption and solubility, resistance to wear, minimum irritation to tissues, and cariostatic action [33].

In this study, maxillary first premolars extracted for orthodontic purpose, which were free of caries, developmental defects, enamel micro-fractures and discoloration were included.

Deionized water has no effect on the protein structure and neither does it alter the enamel structure so it was used as a storage solution for extracted teeth.

Pumice prophylaxis was used for cleaning the occlusal surfaces of premolars prior to etching. *Blackwood et al.,2002* showed that between enameloplasty, air abrasion and pumice prophylaxis, the least microleakage was seen with the conventional pumice prophylaxis [34].

The first generation of Flowable composites was introduced in 1996 [35]. The low viscosity of Flowable composites can be obtained either by decreasing the percentage of fillers or modifying the resin monomers[36].Their lower filler loading results in greater polymerization shrinkage and lower mechanical properties compared to hybrid composites [37].

Flowable composites are suggested by manufacturers for various indications (pit and fissure sealant, repair of marginal defects, liners in deep cavities). Huge differences are observed in the viscosity and flow characteristics of flowable resin composites that can have a potential influence on their clinical behaviour during handling and thus on their clinical indications [38].

However, manufacturers claim many advantages for these new materials. Their low modulus of elasticity and increased flow capacity might provide more contraction stress relaxation and could reduce the frequency of marginal microleakage and possible de-bonding [39].

Vertise Flow is the first self-adhering resin composite including in its formulation the OptiBond technology. It is the logical continuum in the chain of product development aiming towards simplification and ease of application. The bonding mechanism with tooth structure is a chemical bonding achieved between phosphate functional groups of Glycerophosphate dimethacrylate (GPDM) monomers and calcium ions of enamel and dentine. Vertise Flow eliminates the additional steps of etching/priming/bonding, otherwise necessary to bond a resin composite to dentin and enamel. Moreover, it offers favourable mechanical and physical properties [36].

Timing of sealant placement is critical. Newly erupted teeth are the ones that are most susceptible candidate for carious attack and hence need the protection of their pits and fissures. But isolating them is the most difficult. Until few years the only moisture tolerant sealants were glass ionomers [40]. Their mechanism of adhesion is ionic bonding, not micromechanical retention to an acid etched enamel surface. *Paradi and co workers, 2003* reported low sealant retention rates with glass ionomer cement [41].

Resin-based sealant technology has introduced a moisture-tolerant chemistry. While traditional sealants are hydrophobic, Embrace WetBond is hydrophilic. On light-curing, this sealant has physical properties similar to other commercially available sealants [5]. Embrace WetBond incorporates di-, tri- and multifunctional acrylate monomers into an advanced acid-integrating chemistry that is activated by moisture. When applied to etched plot dried enamel surface, which does not show the typical frosted chalky white appearance, the sealant spreads over the enamel surface.

Because of its unique chemistry, Embrace WetBond is miscible with water and flows into moisture-containing etched enamel and combines with it [5]. Additionally, a longitudinal study done *in vivo* with Embrace WetBond has shown a retention rate of 90%. Only 32 teeth out of the 334 teeth in the study required replacement of the sealant [42].

In the present study, a qualitative technique of dye penetration was used for detection of leakage. Penetration of dye indicates the lack of a perfect seal. The detection of the dye in the under penetration zone of the etched enamel indicate a susceptible leakage pathway. Clinically, this may imply that the remaining etched area could be a factor for development of caries by microleakage, if the sealant was partially or completely lost.

Moreover, evaluating the microleakage and recently the nanoleakage represent a refined means of assessing the defective adaptation of materials[43].Although the use of silver nitrate dye was effective in detecting the nanoleakage [44], ammoniacal silver nitrate dye was used in this study to avoid the deminerlizing effect of regular silver nitrate dye[45].

The SEM was used to measure the nanoleakage and the length of resin tags as the SEM can produce very high-resolution images of a sample surface, and reveal details about less than 1 to 5 nm in size. Due to the very narrow electron beam, SEM micrographs have a large depth of field yielding a characteristic three-dimensional appearance useful for understanding the surface structure of a sample [46].

SEM images (Figures 1-3) indicated the presence of nanoleakage in all samples sealed with different Pit and Fissure Sealants as manifested by the infiltration of silver ions at the sealant/enamel interface.

These findings could be attributed to the effect of resin contraction at the time of its polymerization [47]. In addition, the improper wetting of the resin to enamel and bonding resin viscosity [48].The previously nominated factors could create nanogaps between the bonding resin and dentin surfaces. Confirming the same assumption, other researchers had related the occurrence of nanoleakage to the presence of areas of imperfect resin infiltration, retained water or other solvent, poor polymerization, or phase separation [48, 49].

Complete penetration of sealant into complex fissure systems is difficult due to the phenomenon of closed end capillaries or isolated capillaries. Some lateral fissures arising from the main fissures also fail to be filled with sealant [50].

On the other hand, various degrees of silver penetration and brightness were evident between samples of different groups, Fig (1-3) indicating different degrees of nanoleakage. Nanoleakage is significantly less in Vertise Flow than the other two sealants which were not significantly different from each other ($p < 0.05$).This result could be related to the chemical nature of the adhesive systems themselves that is reflected on the expected degree of polymerization shrinkage and the degree of water sorption (dye solution).

In the present study the mean length of resin tags, Figs. (4-6) obtained was in the range of approximately 154 µm to 291µm. Other studies [51-53] reported means with less value than measurements obtained. Appropriate method of application of sealants and viscosity of the sealant, are also factors influencing the microleakage, and if a proper application method is followed, it can increase the length of resin tag and thus improve the efficiency of the sealant in preventing caries [51].

It was observed in our study that, the longer the resin tags of a sealant the lesser is the nanoleakage and consequently the better is its sealing ability.

It should be noted that the results of the present study are valid for *in vitro* conditions. Depending on the environment, all Pit and Fissure Sealants may act differently due to other variables like type of fissures, preparation of fissures, enamel etching and conditioning, application of bonding agent and contamination of prepared surfaces.

Appropriate method of application of sealants and viscosity of the sealant, are also factors influencing the microleakage, and if a proper application method is followed, it can increase the length of resin tags and thus improve the efficiency of the sealant in preventing caries.

5. Conclusion

This study clearly indicated that, there does exist a relationship between the length of resin tags and nanoleakage; the longer the resin tags, the lesser the nanoleakage, the better resin adaptation and the better the cariostatic action of the Pit and Fissure Sealants and Vertise Flow composite as good alternative for sealing pits and fissures is recommended. It showed the longest resin tags penetration and the best sealing ability to etched enamel surface.

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