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In Vitro Microleakage Comparison of Flowable Nanocomposites and Conventional Materials Used in Pit and Fissure Sealant Therapy

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ABSTRACT

Objectives: Pit and fissure sealants are recognized as an effective preventive approach in pediatric dentistry. Composite resin is the most commonly used sealant material. Adding nanoparticles to composite resin could result in production of flowable composite with higher mechanical properties and better flowability than previous sealants. This study aimed to compare the microleakage of a flowable nanocomposite and materials conventionally used as pit and fissure sealants.

Materials and Methods: A total of 185 extracted mandibular third molar teeth were selected and randomly divided into 5 groups (n=36): flowable nanocomposite, flowable composite, filled sealants, nano-filled sealants, and unfilled sealants. Five teeth were reserved for examination under a scanning electron microscope (SEM). The samples were thermocycled (5-55°C, 1-minute dwell time) for 1000 cycles and immersed in 0.2% fuchsine solution for 24 hours. Teeth were sectioned buccolingually. Microleakage was assessed qualitatively and quantitatively by means of dye penetration and SEM. Data were analyzed using chi-square, Kruskal-Wallis, and Bonferroni-corrected Mann-Whitney U tests.

Results: Qualitative microleakage assessment showed that flowable composite and nanofilled flowable composite had almost no microleakage (P<0.001). Regarding quantitative scores, the nanofilled flowable composite and unfilled fissure sealant showed the lowest and the highest rate of microleakage, respectively. No statistically significant difference was found between the two flowable composites (P=0.317). Filled resin-based sealant had significantly lower microleakage than unfilled resin-based sealant (P<0.001).

Conclusion: Use of flowable and nanofilled flowable composites (but not unfilled resin-based fissure sealant) is recommended for sealing of pits and fissures of molars.

Keywords: Dental Leakage; Pit and Fissure Sealants; Composite Resins; Microscopy, Electron, Scanning

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INTRODUCTION

Dental caries is among the most common diseases in children and adolescents; about 21% of children aged 6 to 11 years and 58% of children aged 12 to 19 years have experienced dental caries in their permanent teeth [1]. Occlusal surfaces of permanent teeth may have deep pits and fissures that can trap debris and microorganisms and therefore have an increased risk of developing dental caries [2]. Occlusal carious lesions have been observed in up to 66% of patients [3-5]. Pit and fissure sealants are recommended to prevent occlusal caries in permanent teeth [6]. The inhibiting properties of sealants are attributed to physical blockage of the pits and fissures, which precludes accumulation of bacteria and inhibits the leakage of fermentable carbohydrates into the pits and fissures [2].

A wide range of materials are used as sealants, but composite resin is the most commonly used sealant material. Fissure sealants are divided into three major groups based on their composition: polyacid-modified resin sealants, glass ionomer sealants and resin-based sealants. Glass ionomer sealants are available in two forms of conventional and resin-modified glass ionomers. Resin-based sealants are grouped based on different characteristics such as their polymerization mechanism. Accordingly, they can be divided into four groups of ultraviolet-light polymerizing (which is now outdated), auto-polymerizing, light-curable, and fluoride-releasing types. They also can be opaque or transparent. They may also be filled or unfilled [7-10]. Compared to no-sealant controls, using sealants has been shown to reduce caries formation, especially if resin-based sealants are used (but not glass ionomer sealants) [11,12]. However, evidence regarding effectiveness of resin-based sealants compared to fluoride varnishes is low [13]. Many studies have compared the caries-prevention effects of resin-based versus glass ionomer sealants; there might not be a considerable difference between these classes of sealants [14-16]. Comparison of the cariostatic efficacy of glass ionomers with that of resin-modified glass ionomers or polyacid-modified resin sealant, or between polyacid-modified resin sealant and resin-based sealants have revealed no significant difference [9]. Microleakage of glass ionomers has been tested, and some certain brands (such as Fuji IX) have been preferred over others [17,18]. Ovrebo and Raadal [19] compared leakage of glass ionomer (Fuji III) with resin-based sealants, and found considerable leakage in all glass ionomer samples with extensive dye penetration through the glass ionomer-enamel interface or even through the glass ionomer itself. However, they did not observe microleakage in composite resin samples; still, they suggested that glass ionomers might have cariostatic properties due to fluoride release.

Different methods and materials have been proposed to reduce microleakage of fissure sealants. Questionably carious fissures should be opened and caries should be removed prior to sealing [20]. It is also shown that acid etching of the enamel before smearing glass ionomer decreases the microleakage [21]. Pakdel et al. [22] assessed the effect of fissurotomy before sealant application, and found positive results. Tehrani et al. [23] compared outcomes of different treatments [i.e., acid-etching + fissure sealant (conventional method), acid etching + bonding agent (Single Bond) + fissure sealant; self-etching primer + bonding agent (SE Bond) + fissure sealant, acid-etching + bonding agent + flowable composite (Filtek Flow); and self-etching primer + bonding agent + flowable composite]. They found that acid etching plus application of a bonding agent prior to application of conventional sealant or flowable composite to be the most effective method [23]. Bagherian et al. [24] compared the efficacy of fissurotomy bur + acid etching, pumice prophylaxis + acid etching and acid etching alone and concluded that fissurotomy together with pumice prophylaxis accompanied by acid etching might be the best option among the tested three. Lupi-Pegurier et al. [25] tested the effect of Er:YAG laser conditioning on microleakage of pit and fissure sealants, and reported that Er:YAG laser alone is not effective and cannot be a substitute for pre-sealing etching. The marginal sealing ability of sealant materials plays an important role in their cariostatic efficacy. If microleakage occurs at the sealant-enamel interface, bacteria and other molecules can pass through this area and lead to development of caries [10, 26]. One of the most cited methods to evaluate the efficacy of sealants is microleakage assessment at the tooth-sealant interface [27].

One of the latest innovations in the field of composite resins is the use of nanotechnology. Adding nanoparticles to composite resins could allow for production of flowable materials with better mechanical properties and flowability than previous sealants [10]. New types of nanofilled composites have a filler content of more than 70% and despite this high filler content, they have good flow. The aim of this in vitro study was to compare the microleakage of flowable nanocomposites and conventional materials used as pit and fissure sealants.

MATERIALS AND METHODS

A total of 185 intact and caries-free permanent mandibular third molars with deep developmental pits and fissures on their occlusal surfaces (extracted for orthodontic reasons or because of periodontal disease) were selected for this study. If the samples had a fluorescence score of more than 20 using DIAGNOdent (Feist Siegert Dental, Onalaska, WI, USA), they were considered carious and excluded from the study [28,29]. Teeth had to be free from caries, cracks, hypoplasia, restorations, or fissure sealants. Also, they had to have deep grooves requiring fissure sealants (deep and narrow grooves such that their floor could not be seen under dental light). The teeth were debrided using hand scalers and cleaned with a low-speed prophylaxis brush and stored in 2% thymol until the experiment (shorter than 6 months of storage). The samples were randomly divided into 5 groups (n = 36) according to the sealant material used for pit and fissure sealant and a number of 5 teeth were stored for examination under a scanning electron microscope (SEM). The study protocol was approved by the ethics committee of the university (6473521).

The experimental groups were as follow:

Group 1. Unfilled resin-based pit and fissure sealant (Clinpro; 3M ESPE, St. Paul, MN, USA)

Group 2. Nano-filled resin-based pit and fissure sealant (Grandio Seal; VOCO, Cuxhaven, Germany)

Group 3. Filled resin-based pit and fissure sealant (Helioseal F; Ivoclar Vivadent, Schaan, Liechtenstein)

Group 4. Flowable composite resin (Heliomolar Flow; Ivoclar Vivadent, Schaan, Liechtenstein)

Group 5. Nano-filled flowable composite resin (Filtek Z350 XT; 3M ESPS, St. Paul, MN, USA)

The materials used in this study and their ingredients are presented in Table 1.

Table 1. Materials used in this study and their ingredients

Brand	Composition
Etch.Rite	%38 phosphoric acid gel
Adper Single Bond 2 Adhesive	Bis-GMA, HEMA, dimethacrylates, ethanol, water, novel photo-initiator system, methacrylate functional copolymer of polyacrylic, poly-itaconic acids
Filtek Z350XT Flowable	The monomer matrix consists of Bis-GMA, triethylene glycol methacrylate, procrylat resins The fillers are ytterbium trifluoride, silica filler, zirconia/silica cluster filler (65wt%)
Heliomolar Flow	The monomer matrix consists of Bis-GMA, urethane, dimethacrylate, triethylene glycol methacrylate (40.5 wt%) The fillers are silicon dioxide, ytterbium trifluoride, copolymer (59wt%); Additional contents are catalysts, stabilizers and pigments
Helioseal F	The monomer matrix consists of Bis-GMA, urethane dimethacrylate, triethylene glycol dimethacrylate (58.6wt%) Fillers are silicon dioxide and fluorosilicate glass (40.5wt%); Additional contents are titanium dioxide, stabilizers and catalysts
Grandio Seal	The monomer matrix consists of Bis-GMA and TEGDMA Inorganic fillers (70wt%)
Clinpro	The monomer matrix consists of Bis-GMA, triethylene glycol dimethacrylate Reinforced inorganic filler Components of the photo-initiator system consist of ethyl 4-benzoate, diphenyliodonium hexafluorophosphate, DL-camphorquinone; TBATFB fluoride releasing source; Rose Bengal sodium adds color before curing

Before sealant placement, enameloplasty was performed for all occlusal fissures using a fissurotomy bur (SS White, Lakewood, USA) and a high-speed handpiece (Pana-air; NSK, Tokyo, Japan). The occlusal surface was dried with cotton pellets and etched with 37% phosphoric acid gel (Etch-Rite; Pulpdent Corporation, Watertown, MA, USA) for 20 seconds and the gel was rinsed off with water for 20 seconds. Then, the samples were dried with oil-free compressed air, and evaluated for frosty enamel appearance after etching.

Two layers of a bonding agent (Adper Single Bond 2; 3M ESPE, St. Paul, MN, USA) were applied and after air-thinning, were light-cured for 20 seconds at 650 mW/cm2 with a LED light curing unit (Woodpecker, Beijing, China). All sealants were in the form of syringe. The sealants were applied in accordance with the manufacturer's instructions. To prevent void formation, the sealant material was applied from one side of the occlusal surface, and gently guided by a dental explorer into all occlusal grooves. Each specimen was light-cured for 60 seconds (20 seconds from each of the occlusal, buccal, and lingual directions). Then, the samples were immersed in distilled water, incubated (37°C, 24 hours) and thermocycled (SD Mechatronics Thermocycler; SD Mechatronik GmbH, Westerham, Germany) for 1000 cycles between 5-55°C, with a dwell time of 30 seconds in each bath and a transfer time of 15 seconds. Before dye penetration test, the root apex was sealed with sticky wax and the teeth were covered with two layers of nail polish except for 1 mm margin around the sealant. The teeth were placed in 2% basic fuchsine dye for 24 hours at room temperature. The teeth were washed several times by water, and the roots were cut off from the crown at the cementoenamel junction. After drying, the samples were mounted in auto-polymerizing acrylic resin. Three buccolingual cuts were made using a 1-mm diamond saw working at 1000 rpm and cooled with water (Nemo, Tehran, Iran) creating 6 cross sections per tooth for analysis (a total of 1080 sections were assessed for microleakage). The sections were coded by someone not involved in the study. The sections were observed under a stereomicroscope at ×50 magnification (Dino-lite, New Taipei City, Taiwan). Dye penetration was once evaluated qualitatively by two blinded observers, according to the following criteria for microleakage, on the section showing the highest amount of microleakage. Disagreements were settled through discussion; 0 = no dye penetration; 1 = dye penetration into the superficial one-third of sealant-enamel interface; 2 = dye penetration into the middle one-third of sealant-enamel interface; and 3 = dye penetration into the deep one-third of sealant-enamel interface (Fig. 1). Presence or absence of voids were checked in sealant of each tooth. Dye penetration was also assessed quantitatively. In each tooth, the section showing the highest microleakage was chosen, and microleakage was calculated using modified Duangthip and Lussi scale [30].

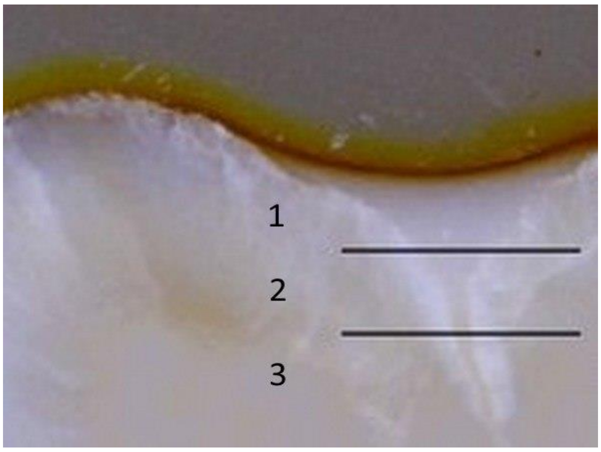

Fig. 1: Qualitative scores of dye penetration

The ratio of the summation of dye penetration in the buccal and lingual groove walls (in millimeters) to the total tooth-sealant interface in the buccal and lingual groove walls (in millimeters) was calculated for each tooth and analyzed using image analysis software (Image J, NIH, Washington DC, USA).

Scanning electron microscopy:
In each group, one tooth was allocated for SEM evaluation. Each tooth was given two buccolingual cuts to make a section of 2 mm diameter and the surface was polished using 2000-grit carbide abrasive paper. The sections were prepared for SEM analysis according to the Gateva's study [31].

Statistical analysis:
Sample size was determined based on the results of Singh and Pandey [32] in order to obtain a power of 80%. Descriptive statistics were reported. Cohen's Kappa was used to estimate interobserver agreement for qualitative microleakage assessments, which indicated a high agreement (Kappa=0.842, P=0.001). Qualitative dye penetration results were compared using the chi-square test. Also, presence of void among the five groups was compared using the chi-square test. Due to the lack of normality of quantitative microleakage data (Kolmogorov-Smirnov test), the Kruskal-Wallis and Mann-Whitney U tests with a Bonferroni correction were used for analyses via SPSS version 20 (SPSS Inc., IL, USA). Level of significance was set as 0.05 for Kruskal-Wallis and chi-square tests, and was adjusted to 0.005 using the Bonferroni method, for Mann-Whitney U test.

Qualitative assessment:

Groups 4 and 5 almost had no microleakage while group 1 had the highest microleakage score. Comparison of the qualitative microleakage scores of the five groups using chi-square test showed a significant difference (P<0.001, Fig. 2). Number of teeth with voids in their sealants in groups 1 to 5 was 30, 24, 10, 10, and 7, respectively. Chi-square test showed a significant difference in this respect among the 5 groups (P<0.001).

RESUTS

Quantitative assessment:

The mean microleakage scores of all groups are listed in Table 2. Teeth sealed with Filtek Z350 (group 5) showed the lowest microleakage score, and Clinpro (group 1) had the highest microleakage score among all five groups. The Kruskal-Wallis test showed statistically significant difference in this respect among the groups (P<0.001, Fig. 3).

Intergroup comparisons revealed statistically significant differences (P<0.005) between group 1 compared to groups 3, 4 and 5, and between group 2 compared to groups 4 and 5.

SEM observations:

Evaluation of the sealant-enamel interface using SEM images at ×1500 and ×3000 magnification indicated no difference between the groups in terms of shape and depth of the formed resin tags (Figs. 4 to 8).

DISCUSSION

A variety of materials are used for sealant treatment, but resin-based sealants are the most commonly used [6,7,35].

Table 2. Mean ± standard deviation microleakage score of different groups (n=36 per each group)

Group	Microleakage score
1	0.18 ± 0.24
2	0.129 ± 0.23
3	0.03 ± 0.08
4	0.00 ± 0.03
5	0.00

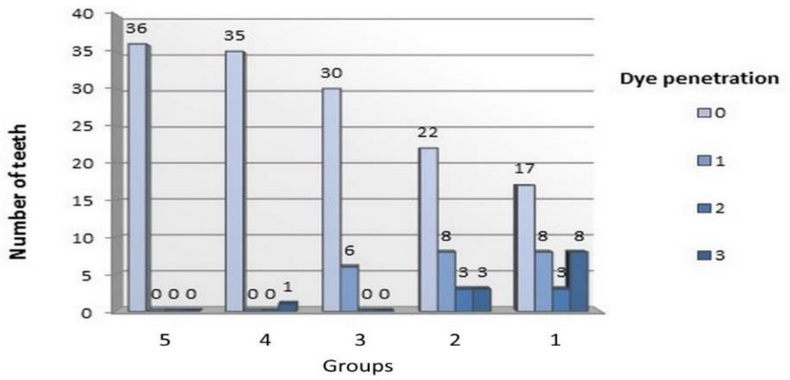

Fig. 2: Distribution of qualitative scores of dye penetration in different groups

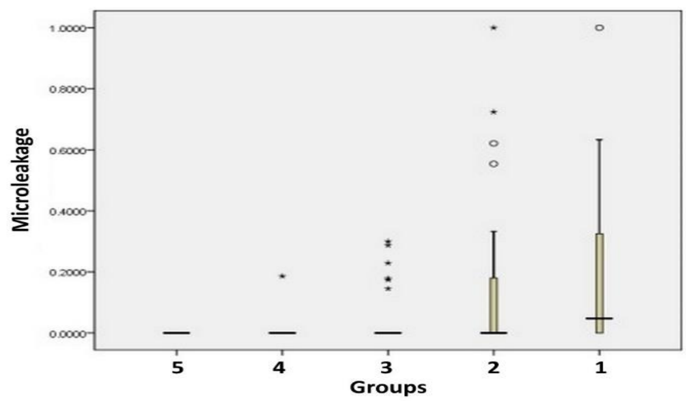

Fig. 3: Quantitative dye penetration values in different groups

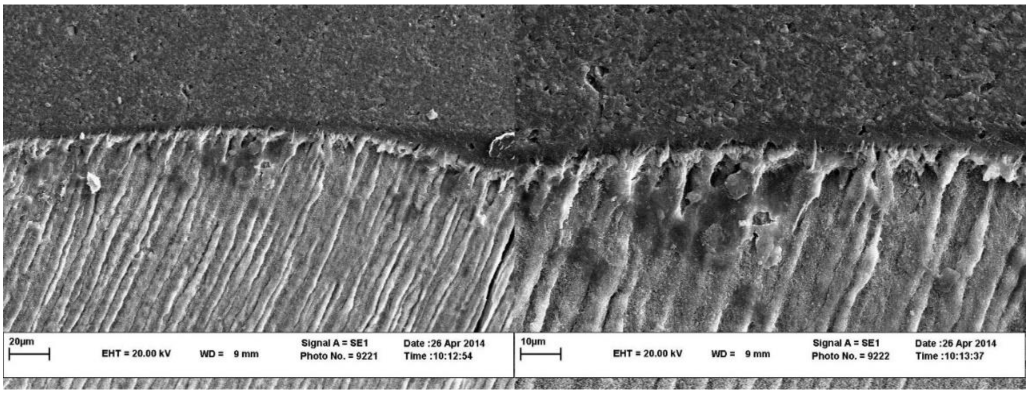

Fig. 4: SEM image of nanofilled flowable composite. Left: x1500, Right: x3000

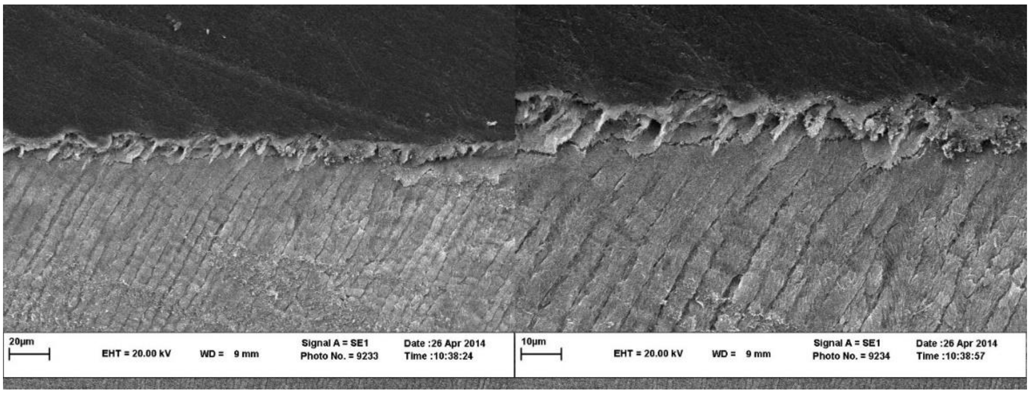

Fig. 5: SEM micrograph of flowable composite. Left: x1500, Right: x3000.

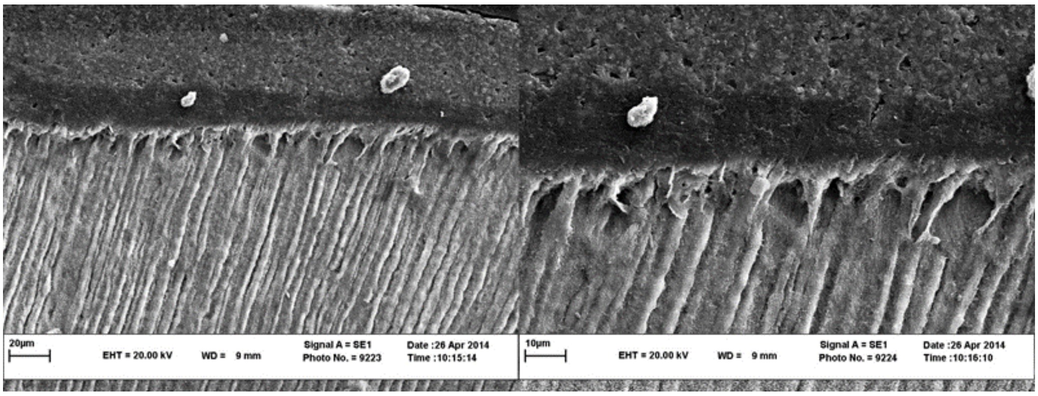

Fig. 6: SEM image of filled fissure sealant. Left: x1500, Right: x3000.

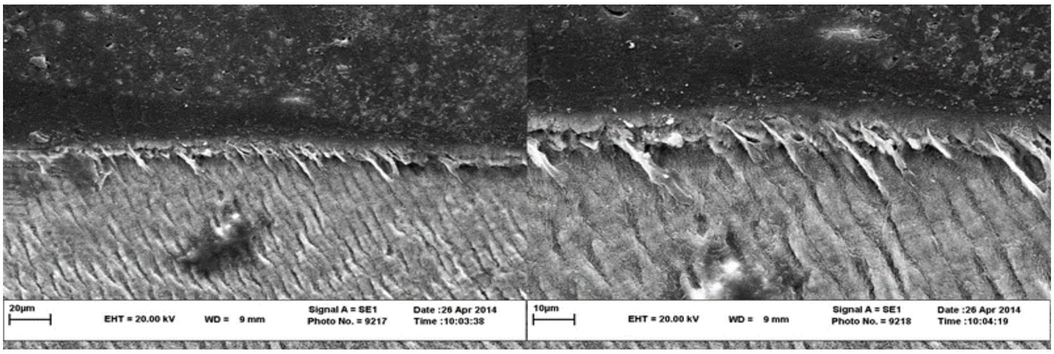

Fig. 7: SEM micrograph of nanofilled fissure sealant. Left: x1500, Right: x3000.

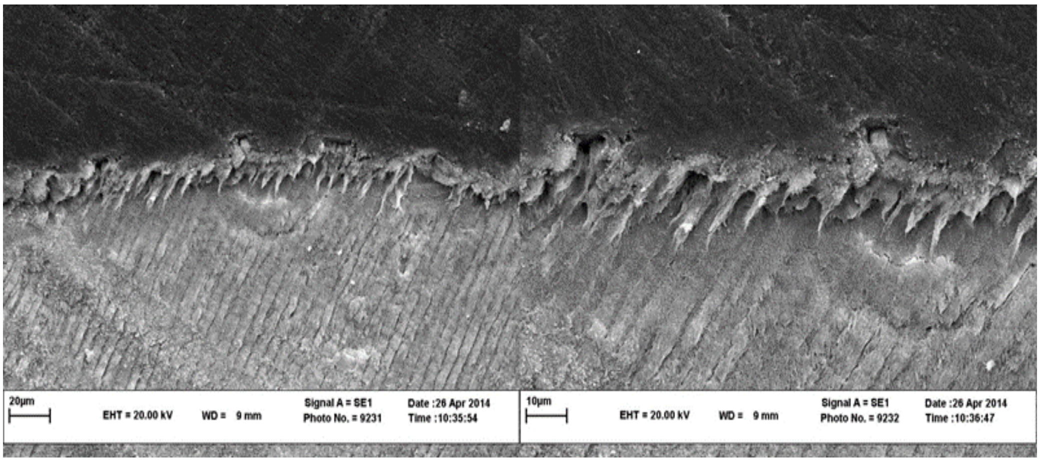

Fig. 8: SEM image of unfilled fissure sealant. Left: x1500, Right: x3000.

The caries preventive effect of resin-based sealants depends on sealing of the grooves by micromechanical bond of the materials to the acid-etched enamel surface [36].

Some studies have reported that the sealing ability of resin-based sealants is related to providing a physical barrier that prevents metabolic exchange between cariogenic microorganisms in the fissures and the oral cavity [37,38].

Thus, the efficacy of sealants for caries prevention depends on adaptation and long-term retention of sealant material [38]. Flowability is another important characteristic of sealants, since penetration depth is dependent on viscosity. Adding nanoparticles to composites has resulted in materials with better mechanical properties and flowability [10]. In the present in-vitro study, microleakage of flowable nanocomposites and four conventional materials used as pit and fissure sealants was compared. In the present study, enameloplasty was performed for the occlusal pits and fissures of the samples using a fissurotomy bur and a high-speed handpiece. Enameloplasty improves retention, adaptation and penetration of sealant into the fissures [39,40]. Before placement of sealants, the occlusal surface was treated with 37% phosphoric acid followed by application of a fifth generation bonding agent. According to previous studies, total-etch technique can result in better penetration of sealants and less microleakage [41,42], and the use of dentin bonding agents under sealant material can be beneficial for reducing microleakage and increasing bond strength and retention [43,44].

The samples were thermocycled to simulate thermal stresses that result in formation of marginal gap at the sealant-tooth interface. Dye penetration test was used to evaluate microleakage, since it is inexpensive and nontoxic and is widely used. Also, dye can be detected in low concentrations [45]. In-vitro microleakage evaluation by dye penetration test, is more meticulous compared to what happens in the oral environment [46], since dye penetrates more easily than bacteria and their metabolites. Moreover, accumulation of proteins in the marginal gap at the tooth-sealant interface can improve the seal. Therefore, the results may be overestimated in vitro, and the material may have better clinical performance in the oral cavity compared to its performance in vitro [47]. Raskin et al. [48] showed that in-vitro microleakage evaluation is accurate if each sample is sectioned at least 3 times buccolingually [48]. Therefore, in our study, each tooth was sectioned 3 times in a buccolingual direction creating 6 cross sections for microleakage evaluation. In the present study, microleakage was evaluated quantitatively. This method is objective, and eliminates the need for intra- and inter-observer agreement evaluations [27].

The results of the present study showed that the microleakage of flowable composites (Heliomolar Flow, Filtek Z350) was significantly lower than the 3 types of fissure sealants tested. Gillet et al. [49] examined in vitro microleakage and penetration depth of three types of materials used as sealants: conventional composite, flowable composite, and fissure sealant (Helioseal F). They found that although Helioseal F had the highest penetration depth, it had the

highest microleakage, but both composites used in the study showed no microleakage, although their penetration depth was less. They concluded that using a flowable composite is a superior technique for sealing of caries-free deep fissures [49].
Among three fissure sealant materials evaluated in our study, filled resin-based pit and fissure sealant (Helioseal F) showed the best result and significantly less microleakage compared to unfilled resin-based pit and fissure sealant (Clinpro). This could be due to higher viscosity and filler content of Helioseal F, which result in higher fracture resistance, and less marginal gaps and polymerization shrinkage. These results are consistent with the results of Fernandes et al, [50] and Flangan and Pearson [51]. Several studies in the recent years have documented that the use of bonding agents in total-etch mode, prior to the application of flowable composite resin as sealant improves retention and marginal adaptation [52,53]. Kwon and Park [54] examined the microleakage of three flowable composites (Filtek Flow, Tetric Flow, Charmfil Flow) and a filled sealant (Ultraseal XT Plus), and found that the microleakage of all three flowable composites was higher compared to the filled sealant used. They concluded that the use of the filled sealant is more effective in sealing of mechanically prepared occlusal fissures [54]. These findings are inconsistent with the results of the present study, perhaps because of methodological differences between the studies or differences in materials or even brands used in the two studies.
Although the formation of resin tags is an indicator for sealing ability, some studies have proven a weak correlation between resin tag formation and microleakage [30,52-54]. In the present study, the SEM evaluation of enamel-sealant interface showed resin tag formation in all groups, but no correlation was found between the depth and shape of resin tags and microleakage. This could be due to the use of dentin bonding agents between the tooth and sealant material; therefore, resin tags formed in all groups were due to the penetration of bonding agent into the etched enamel and the sealant material adhered to the bonding agent.

CONCLUSION

Use of nano-filled flowable composites followed by flowable composites is advantageous in terms of low microleakage after sealing of occlusal fissures, especially compared to unfilled resin-based pit and fissure sealant, which might have the highest microleakage among the tested brands and material types.

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