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ORIGINAL RESEARCH

Evaluating the effect of three fissure preparation techniques on microleakage of a colored flowable composite used as a fissure sealant

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Abstract

Pit and fissure sealants play an essential role in preventive dentistry. This study evaluates the microleakage levels of a new and colored flowable composite applied as a sealant after three preparation techniques. A total of 24 non-carious mandibular permanent molars with deep pits and fissures were included in the study. Pit and fissures were prepared with 37% phosphoric acid, tungsten carbide bur and fissurotomy burs (SS WHITE Dental, New Jersey, USA) using conventional, enameloplasty and fissurotomy techniques. All samples were thermocycled following the placement of Rainbow Flow (PPH CERKAMED Wojciech Pawłowski, Poland) as a sealant and sections were taken after immersion in methylene blue dye. The microleakage levels of the samples were examined under a stereomicroscope (Olympus SZX-7 Olympus SZ-61 Stereo Microscope) at 2.8× magnification to analyze the dye penetration of the flowable composite. The 144 sections were examined and scoring for microleakage was done by examining the dye penetration from the occlusal border to the base of the fissure. Considering all sections regardless of the preparation technique, it was found that 16.6% of the sections have no leakage. Regarding the microleakage scores, the mean score of the conventional group was 1.87 ± 0.98, the mean score of the enameloplasty group was 1.88 ± 1.14, and the mean score of the fissurotomy group was 1.81 ± 1.1. The median scores of the conventional, enameloplasty and fissurotomy groups were 2, 2 and 1.5, respectively. The present study reports no difference between the microleakage level of a colored flowable composite material used as a pit and fissure sealant following three fissure preparation techniques and supports the clinical use of this material.

Keywords

Colored composite; Fissure sealants; Methylene blue; Microleakage; Preventive dentistry

1. Introduction

Tooth decay is one of the most common diseases in childhood and adolescence [1]. The incidence of tooth decay is gradually increasing as a result of changes in eating habits, and it persists as a significant public health problem [2]. Pit and fissure sealant technique is one of the protective methods used to prevent decays that commonly occur on the occlusal surfaces of permanent teeth [3]. These applications provide the physical sealing of pits and fissures, with the goal of preventing the accumulation of bacteria and fermentable carbohydrates in those areas [4].

The thermal expansion coefficient of sealants is 2–4 times higher than that of enamel; consequently, temperature changes in the oral cavity cause cavities to form at the interface between the sealant and enamel. Microleakage, defined as the entry of bacteria and oral fluids into the space between the tooth and the restorative material, affects the survival of sealants by causing bacterial invasion and secondary caries [5, 6]. *In vitro* studies enable evaluation of marginal leakage and estimation of the marginal sealing ability of different materials used as pit and fissure sealants. Given that many new materials have come onto the market, the physical properties and application technique of fissure sealants should be evaluated [7].

Currently, many materials are used as pit and fissure sealants, such as glass ionomer cements, dental compomers, traditional resin-based fissure sealants and flowable composites [8, 9]. Flowable composite materials have become widely used as pit and fissure sealant in the last two decades due to their favorable characteristics, including low viscosity, elasticity modulus and ease of use. The high particle rate of such materials, which are used in lesser thicknesses than resin-based pit and fissure sealants, has been indicated to decrease porosity, increase wear resistance and decrease polymerization shrinkage rates [10–12].

It has been argued that the techniques used to seal pits and fissures are just as important as the materials. Many studies have used different techniques before pit and fissure sealant application with the aim of increasing retention and preventing microleakage [13–15]. Preparation of the fissure by enameloplasty or burs has come to the forefront as a technique that allows deeper penetration of the acid and the sealant and that increases retention by enlarging the surface area; some studies have reported decreased incidence of microleakage in teeth undergoing surface preparation [9, 16]. In contrast, some studies have reported no significant benefit of surface preparation [17–19].

Fear of dentists and a lack of cooperative behavior increase risk of caries in children; hence, dentists may employ a number of methods to obtain a child's cooperation during treatment. One such method is to allow the child to select the color of the restorative material [20, 21]. Colors affect the children's lives psychologically and physiologically [22], and therefore, it is believed that colored restorations could be an effective motivating factor for children visiting a dental clinic [20]. The studies conducted on this subject have investigated the clinical success of colored restorative materials and have suggested that colored restorative materials could be used as an alternative to composite restorations [23, 24].

The present study evaluates and compares the rate of microleakage after the application of a flowable composite-based restorative material with different color options that has recently been introduced in the market as a sealant, which itself follows the preparation of the dental pits and fissures using conventional techniques, enameloplasty and fissurotomy.

2. Materials and methods

2.1 Sample size calculation

The study's power was calculated using power analysis software (G*Power V. 3.1.9.2 program, Universitat Kiel, Germany) [25] to determine the number of samples. On the basis of data from previous studies [26, 27], the calculations showed that 8 samples per group were necessary to provide statistical significance ($\alpha = 0.05$) at 80% power.

2.2 Randomization

Using simple randomization, a blinded operator randomly divided 24 samples into three groups of 8 each. The groups underwent the following treatments:

Conventional Technique (Group A): 37% phosphoric acid;

Enameloplasty Technique (Group B): A 1/4 round tungsten carbide bur and a high-speed handpiece (WK-99 LT, W&H Group, Austria);

Fissurotomy Technique (Group C): Micro short conical fissurotomy bur (SS WHITE Dental, Lakewood, NJ, USA) and a high-speed handpiece (TE-97 RM, W&H Group, Bürmoos, Austria).

2.3 Study sample

A total of 24 permanent third molar teeth, extracted for orthodontic reasons and due to periodontal disease, were collected from the Department of Oral and Maxillofacial Surgery, Hamidiye Faculty of Dentistry, University of Health Sciences.

Inclusion criteria:

- Permanent third molar teeth with deep pits and fissures.
- Teeth free of dental caries, cracks or any developmental anomalies.

Exclusion criteria:

- Permanent third molar teeth with shallow pits and fissures.
- Teeth with dental caries, cracks or any developmental anomalies.

The samples were thoroughly cleaned using a scaler and then dried. The surfaces were evaluated for caries by two pedodontists who conducted a careful visual examination under good illumination. Preparation of tooth samples and all laboratory procedures were performed by a single operator, to ensure standardization.

2.4 Preparation of tooth specimens

The teeth were kept in distilled water until the time of the experiment, at which point they were cleaned with a low-speed handpiece (WK-99 LT, W&H Group, Austria) and a thin-haired brush, then rinsed, air-dried in oil-free pressured air, and divided randomly into three groups (n = 8).

Conventional Technique (Group A): The teeth underwent an acid etching procedure for 20 seconds using 37% phosphoric acid, followed by water rinsing and drying with pressured oil-free air.

Enameloplasty Technique (Group B): Pits and fissures were prepared using a 1/4 round tungsten carbide bur to create shallow holes using a high-speed handpiece (WK-99 LT, W&H Group, Austria). Acid etching was then performed for 20 seconds, after which the teeth were rinsed in water and dried in non-pressured oil-free air.

Fissurotomy Technique (Group C): Pits and fissures were prepared using a micro short conical fissurotomy bur (SS WHITE Dental, New Jersey, USA) and a high-speed handpiece (TE-97 RM, W&H Group, Austria) proportionate to the dimension of the bur cap (a depth of 1.5 mm and a cuspal distance of 1/8–1/10). Acid etching was then performed for 20 seconds, after which the teeth were rinsed in water and dried in non-pressured oil-free air.

In all groups, a C-Bond universal bonding agent (Willmann & Pein GmbH, Germany) was applied to the acid-treated surface and cured using a light-emitting diode (LED) curing light (GuillinWoodpecker Medical Instrument Co. Ltd, China) for 20 seconds. The violet option of the six-color Rainbow Flow (PPH CERKAMED Wojciech Pawłowski, Poland) light-curing flowable composite set was applied as a fissure sealant according to the manufacturer's instructions and cured with the same curing light (GuillinWoodpecker Medical Instrument Co. Ltd, China) for 20 seconds (Table 1).

2.5 Laboratory procedure

The samples were kept in distilled water for 24 hours at 37 °C and then subjected to 500 cycles (Dentester Thermal Cycle Device DTS B1, Dentester, Salubris Technica, Istanbul, Turkey) at 5 °C–55 °C with a 15-second dwell time and a 15-second transfer time to simulate temperature fluctuations in the oral cavity. Thermal cycling was utilized to simulate

TABLE 1. The information given about Rainbow Flow (PPH CERKAMED Wojciech Pawłowski, Poland) flowable composite*.

Ingredients	Available package	Application
Methacrylate resins (bis-GMA, UDMA, TGDMA), inorganic filler 62%, pigments, photoinitiator, inhibitor.	• 1 × syringe containing 1 g of the preparation (chosen colour) + applicators. • Super Six: 6 × syringe containing 1 g of the preparation (colours: orange, yellow, green, blue, purple, pink) + applicators.	• Filling cavities in deciduous and permanent teeth as the first layer in Class I and Class II cavities and for filling class V cavities. • Sealing fissures. • For marking the mouth of root canals.

**The information is taken from the website of https://www.cerkamed.com/product/rainbow-flow.*

temperature variations encountered in the oral cavity. In line with the guidelines of the International Standard Organization (ISO) TB 11450 standard, a total of 500 thermal cycles were performed [28]; according to a thorough review article, it is estimated that a 500 thermal-cycle procedure corresponds to approximately six months of oral function. Some studies documented in the literature have indicated that thermocycling does not exert a significant influence on the occurrence of microleakage in dental restorations [29, 30].

The entire tooth surface—other than the sealant-coated fissures and a 1-mm margin around the fissures—were coated with nail polish. The apical foramina of the teeth were occluded with Denfil Flow (Vericom Co. Ltd., Korea) flowable composite resin, and the samples were immersed in 2% methylene blue dye for 48 hours (Fig. 1). After the samples were removed from the dye solution, they were rinsed with tap water to remove any excess dye. The teeth were cut labiolingually using a diamond disk and a hard tissue microtome (Buehler Isomet, Buehler ISomet 1000 precision sectioning saw, Lake Bluff, IL, USA) to create four sections and six surfaces. Then, the samples were examined under a stereomicroscope (Olympus SZX-7 Olympus SZ-61 Stereo Microscope, Olympus Corporation, Tokyo, Japan) at 2.8× magnification, to analyze the dye penetration of the flowable composite.

A double-blind evaluation was conducted by two calibrated operators using the scoring scale defined by Grande [31] (Fig. 2). The microleakage value for each surface was calculated by dividing the total buccal and lingual dye penetration values by the total lengths of buccal and lingual enamel-fissure sealant interfaces.

0: No dye penetration.

1: Dye penetration up to 1/3 of the fissure depth.

2: Dye penetration more than 1/3 but less than 2/3 of the fissure depth.

3: Dye penetration deeper than 2/3 of the fissure depth.

2.6 Statistical analysis

The scores were analyzed using the kappa test to assess reproducibility among the examiners. The Shapiro-Wilk test was used to evaluate the normality of the data. Microleakage scores observed in study groups were summarized as mean, standard deviation (SD), median and range (min–max). For the purpose of non-parametric comparisons of microleakage scores across the study groups, a Kruskal-Wallis test followed by a *post hoc* Mann-Whitney U test was conducted. Data were analyzed using IBM SPSS Statistics for Windows, Version 23.0 (IBM Corp., Armonk, NY, USA). A value of two-sided $p < 0.05$ was considered statistically significant.

3. Results

This *in vitro* study evaluated the incidence of microleakage using the different preparations of conventional techniques, enameloplasty and fissurotomy followed by the application of a new flowable composite material available in a wheel of various colors that makes the treatment more likely to be accepted by children. In the evaluation of microleakage, labiolingual sections were cut from the samples after thermal cycling followed by immersion in the methylene blue dye, and dye penetration from the occlusal margin to the fissure base was examined and scored (Figs. 3,4,5).

A total of 144 sections were examined for dye penetration. With regard to all sections, regardless of the preparation technique, microleakage was not observed in 16 sections (11.1%). The kappa test revealed a substantial agreement of 0.76 between examiners.

The distribution of microleakage scores across the groups and their different preparation techniques are presented in Fig. 6. The mean microleakage scores were 1.87 ± 0.98, 1.88 ± 1.14 and 1.81 ± 1.1 with medians of 2 (min–max: 0–3), 2 (min–max: 0–3) and 1.5 (min–max: 0–3) in the conventional, enameloplasty and fissurotomy groups, respectively. No statistically significant difference was noted between the three groups in the microleakage scores (p = 0.95) (Table 2).

TABLE 2. Intergroup comparison of mean dye penetration scores.

Groups	Mean	Std. Deviation	p*	*Post hoc* pairwise comparison†
Group A	1.87	0.98	0.951	Group A *vs.* Group B 0.753
Group B	1.88	1.14		Group B *vs.* Group C 0.677
Group C	1.81	1.1		Group C *vs.* Group A 0.851

**Kruskal Wallis test; †Mann Whitney U test.*

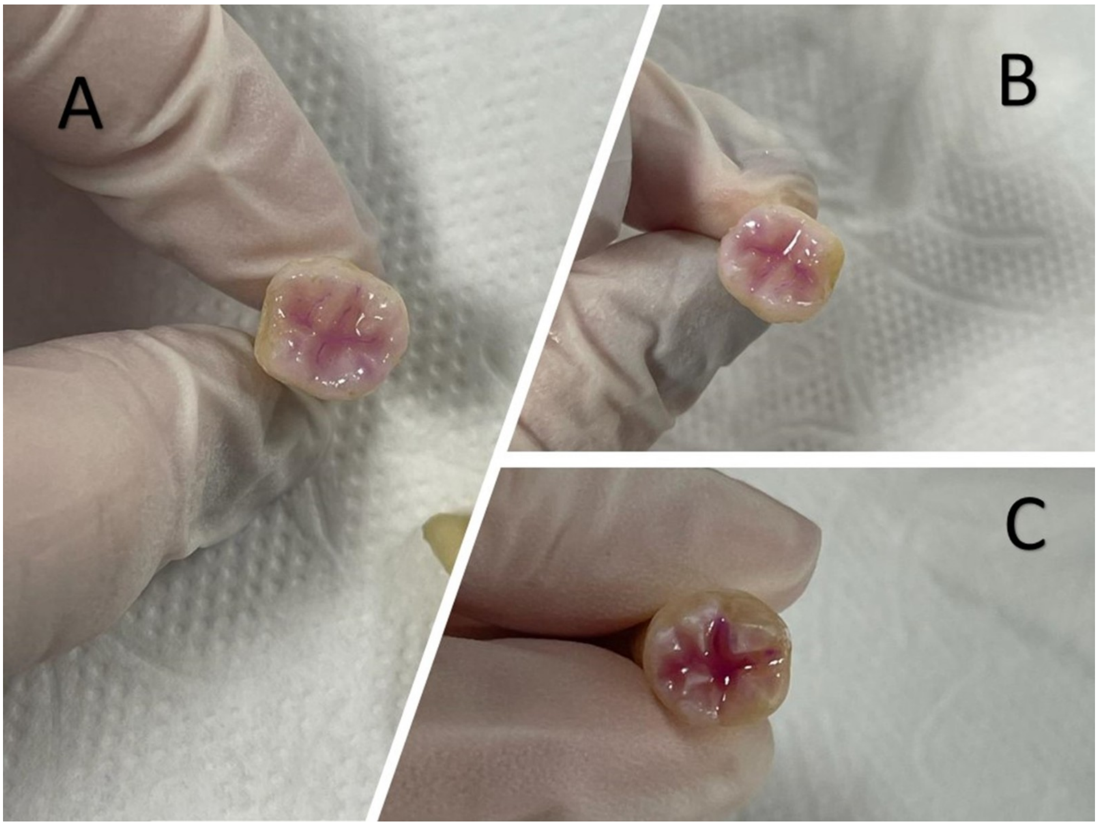


FIGURE 1. Following three preparation techniques, the occlusal fissures of the teeth were occluded with Denfil Flow (Vericom Co., Ltd., Korea) flowable composite resin. (A) Enameloplasty, (B) Conventional, (C) Fissurotomy.

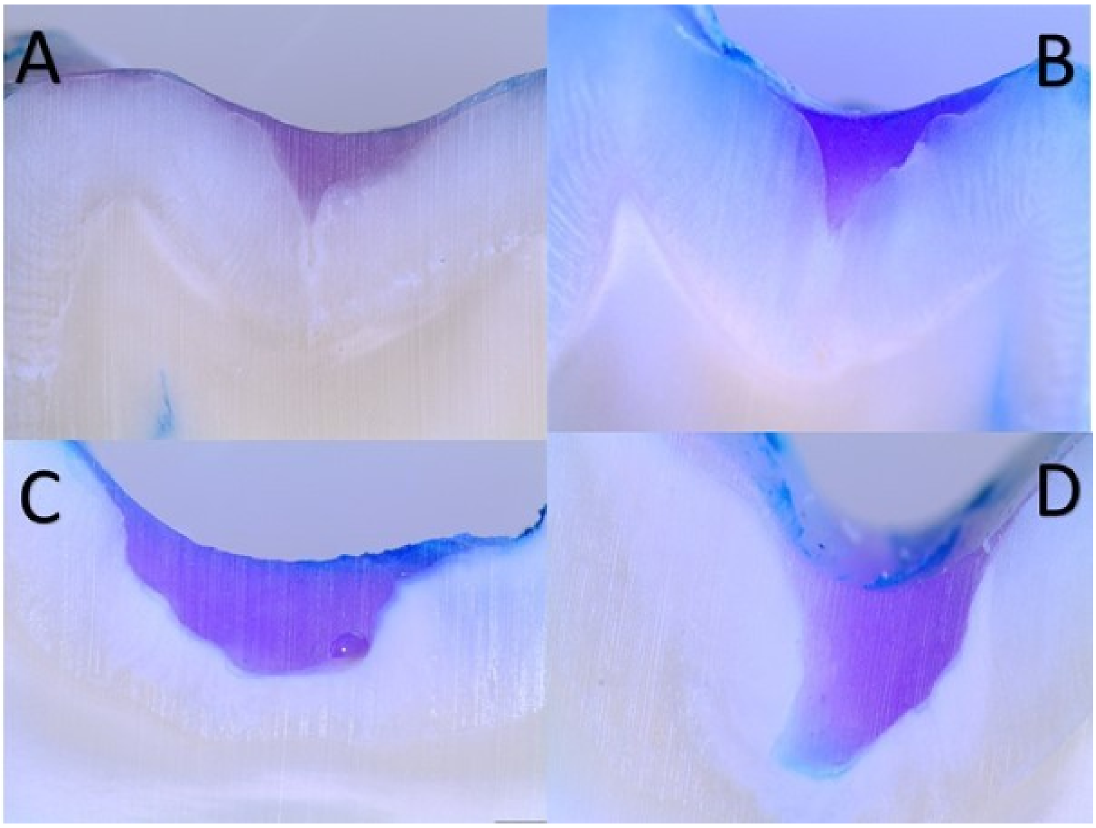


FIGURE 2. Scoring criteria established by Grande. (A) Score 0: No dye penetration. (B) Score 1: Dye penetration up to 1/3 of the fissure depth. (C) Score 2: Dye penetration greater than 1/3 and less than 2/3 the depth of the fissure. (D) Score 3: Dye penetration greater than 2/3 of the fissure depth.

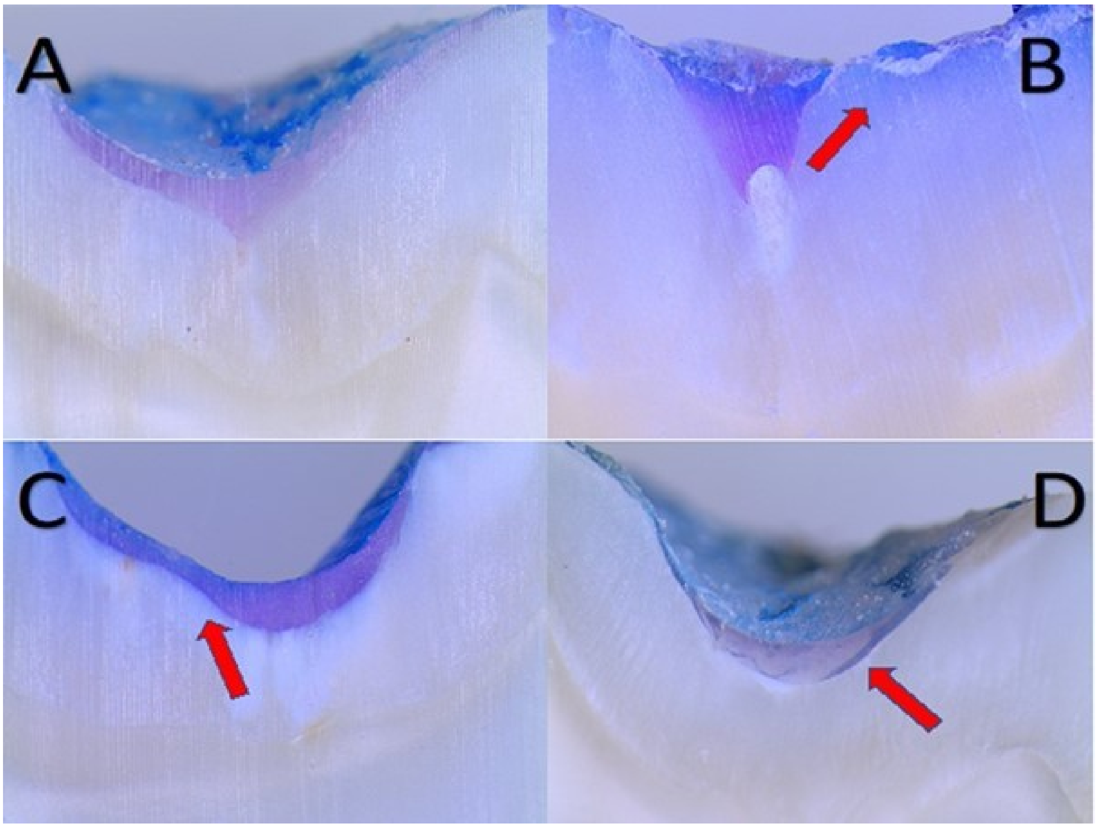


FIGURE 3. Microleakage in conventional fissure preparation group. Microleakage areas are shown in order of A to D (0–3 respectively) with red arrows after conventional fissure preparation technique.

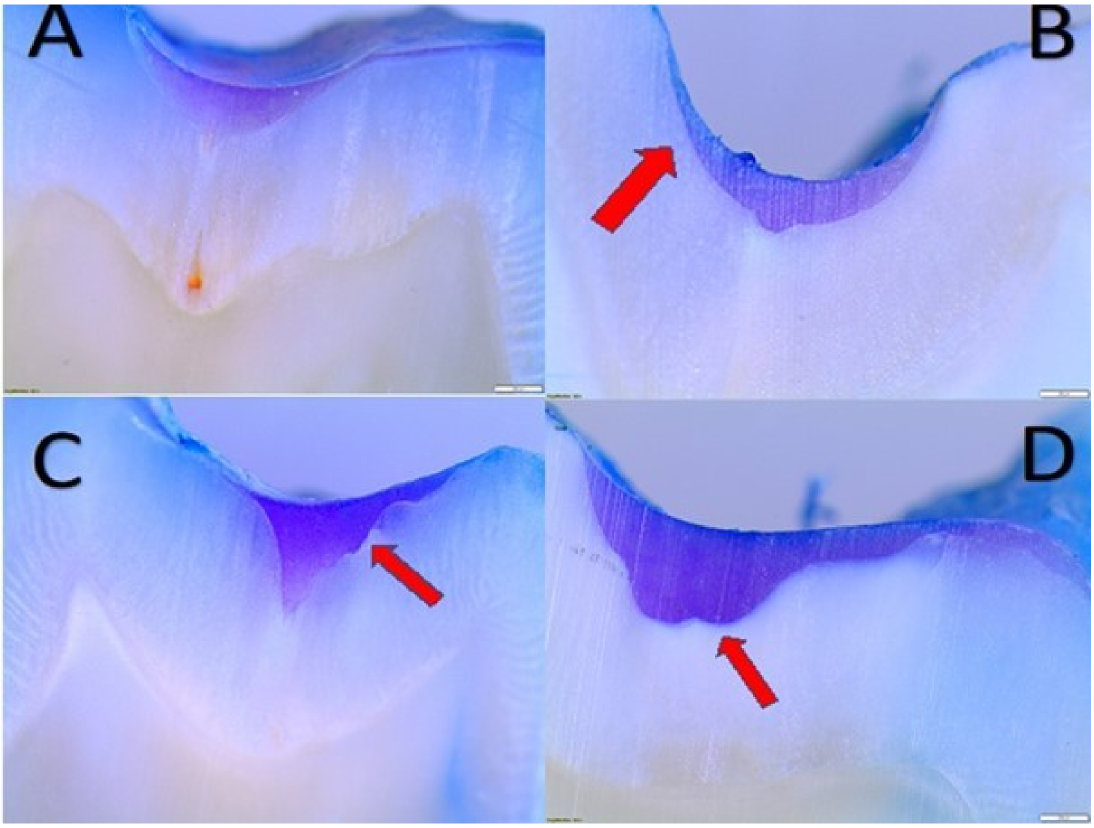
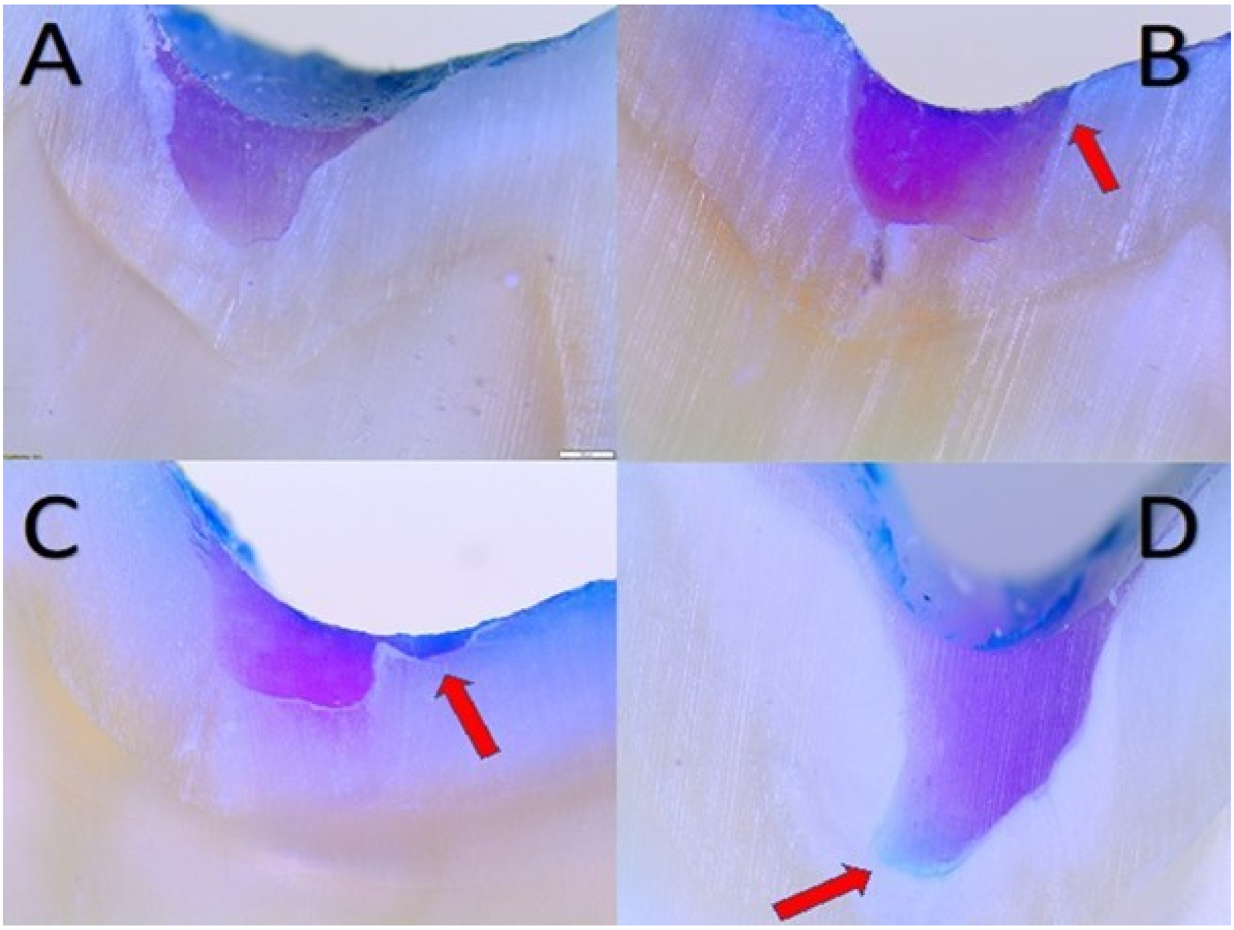
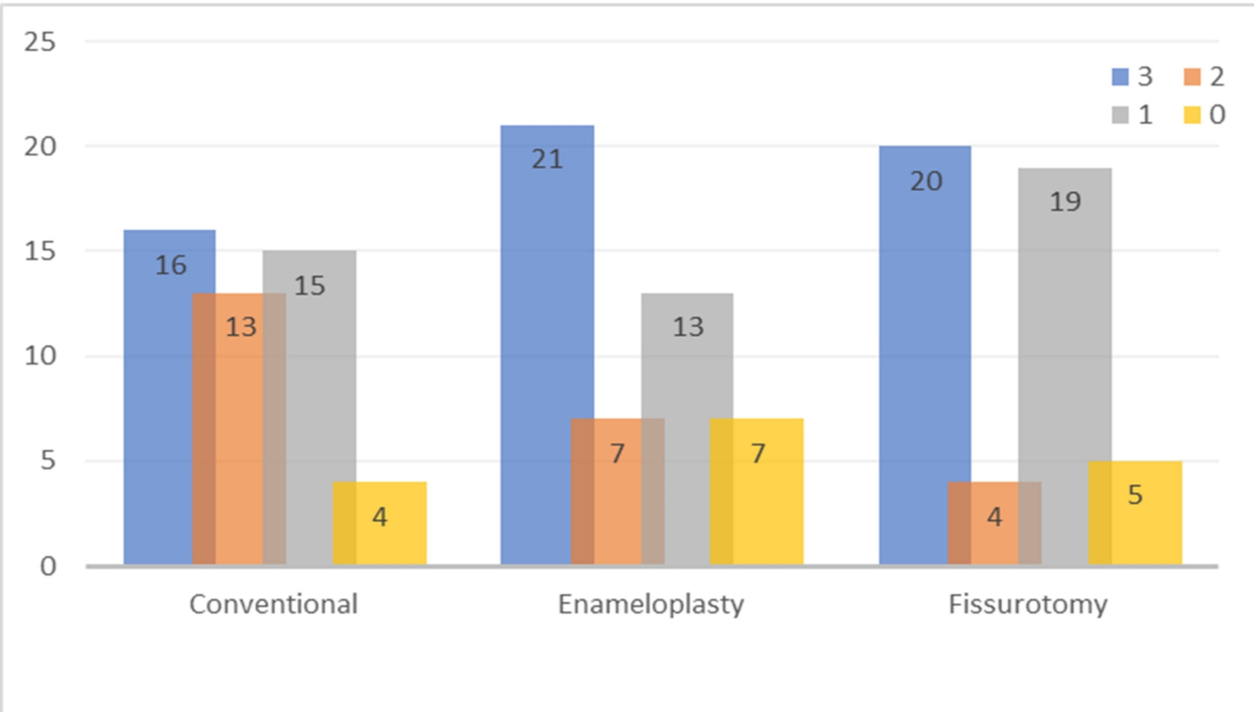


FIGURE 4. Microleakage in enameloplasty fissure preparation group. Microleakage areas are shown in order of A to D (0–3 respectively) with red arrows after enameloplasty fissure preparation technique.



F I G U R E 5. Microleakage in fissurotomy fissure preparation group. Microleakage areas are shown in order of A to D (0–3 respectively) with red arrows after fissurotomy fissure preparation technique.



F I G U R E 6. Distribution of dye penetration scores among three fissure preparation techniques.

4. Discussion

Adequate marginal adaptation is believed to be the most important factor in fissure sealant applications to reduce secondary caries [32]. This study evaluated the microleakage scores of a colored composite material applied with three fissure preparation techniques: conventional, enameloplasty and fissurotomy. The results obtained in this study showed that there were no statistically significant differences between these techniques in microleakage scores.

Many materials have been used for sealing the fissures, including glass ionomers, resin-modified glass ionomers, compomers and flowable composite resins. An ideal fissure sealant has good bond strength and impermeability [33]. A review of the literature reveals that pit and fissure sealant application is effective in preventing caries in children and adolescents, particularly when used together with a bonding agent [33–35].

It has been acknowledged that restorative materials carry the risk of secondary caries formation, and it is argued that this risk can be mitigated by reducing microleakage [36]. In conventional pit and fissure sealant applications, studies have reported higher microleakage when materials are applied without a bonding agent compared to when materials are applied with a bonding agent [37]. Furthermore, studies have indicated that in terms of conventional adhesive systems and self-etch systems, microleakage values tend to be higher for self-etch systems [38, 39].

In light of these factors, this study aimed to compare the microleakage values of a flowable composite material applied with a bonding agent across different preparation techniques. In a study evaluating the performance of flowable composite materials, the rate of microleakage was found to be lower when these were used as a pit and fissure sealant together with bonding agents, compared to other pit and fissure sealant materials [40]. In a study evaluating flowable composite, composite and fissure sealant materials applied to cover fissures, flowable composites were found to have the best performance in terms of penetration depth and microleakage avoidance [41]. In addition to the results obtained by Gillet *et al.* [41] and Dixit *et al.* [42] reported that the incidence of microleakage was lower in groups treated with flowable composite materials in conjunction with bonding agents compared to groups treated without bonding agents Two other studies provided further support for this technique, reporting that the use of pit and fissure sealants in conjunction with bonding agents increases the efficacy of the sealant [43, 44]. It has also been demonstrated that the use of bonding agent substantially decreases the incidence of microleakage, even on a tooth surface contaminated with saliva [17, 45]. In the present study, the application of a bonding agent was followed by the application of flowable composite material; consistent with the other studies [17, 40] examining the incidence of microleakage following fissure sealant application, the incidence of microleakage—although variable across the study groups—was within the acceptable limits.

In a study comparing microleakage rates across colored compomer, ormocer, giomer and resin-modified glass ionomer restorative materials, no significant difference was reported between the colored compomer and other materials in terms of microleakage [46]. Another study compared colored compomers and composite restorations in terms of level of dental anxiety and success of restorations in children undergoing dental restoration; no significant difference was reported between the clinical successes of colored compomers and composite restorations, while the level of dental anxiety was significantly lower in children undergoing treatment with colored compomer material [47]. Therefore, the present study sought to examine the performance of a flowable composite material with different color options (such as blue, purple and yellow) that might make the treatment more likely to be accepted by children. It was hypothesized that clinical use of this material would increase the child's cooperation and acceptance in subsequent sessions.

The tissue-sparing approach aims to preserve the existing dental tissue of young patients and requires the determination of the least invasive treatment possible on the tooth [48]. Minimally invasive interventions are an important approach in the treatment of white spot lesions on teeth, as they preserve the structure of intact teeth, improve aesthetics and improve clinical outcomes of preventive and restorative dentistry [49].

Studies have found that the conventional technique offers the best approach to fissure sealing in the practice of dentistry, because it is simple, quick, inexpensive and requires less equipment [50, 51]. Although the conventional method is commonly used for surface preparation before pit and fissure sealant application, aid etching may not remove pellicles and other remnants may not be removed from the base of the fissures [52]. For this reason, it is debated whether mechanical preparation of the pits and fissures before surface preparation using the conventional method is required or could be used as an alternative to the procedure [53]. Similar to the present research, a study evaluating four different resin- and glass ionomer-based fissure sealants compared the effects of the conventional technique with mechanical preparation methods on the penetration of the sealant; no significant difference was found between the methods [54].

In contemporary dentistry, the preference is to treat caries using minimal invasive approaches. Therefore, instead of removing caries and restoring teeth with fillings, a surface preparation is performed on the enamel, followed by the application of a pit and fissure sealant. Fissurotomy and enameloplasty are frequently the preferred techniques for this purpose [55]. Given the expensive nature of such techniques as laser and microabrasion, it is believed that the accessibility of fissurotomy and enameloplasty makes them more favorable options. In this study, the microleakage values of a colored flowable composite were investigated using different surface preparation techniques.

Some studies in the literature examine enamel preparation techniques prior to the application of a fissure sealant, and they suggest that the increased surface area provides a thicker layer of filling, which in turn provides greater wear resistance [42, 56]. Shapira *et al.* [57] reported that enlarging and deepening the fissures by mechanical preparation increases the bonding strength.

Resin infiltration has been proposed as a new approach in minimally invasive dentistry. It improves the appearance of white spot lesions by impeding enamel demineralization

[58, 59]. In one study, conventional and resin infiltration techniques were used on pits and fissures, and the incidence of microleakage was reported to be lower with the resin infiltration technique [60]. Since resin infiltration is a technique used for lesions of initial caries, it was chosen for this study to compare different techniques for sealing pits and fissures.

Microabrasion is a chemical and micromechanical method that removes a microscopic layer from the enamel surface; it is considered a conservative and non-restorative approach [61, 62]. In a study examining the effect of microabrasion on the microleakage of modified glass ionomer and compomer restorations in class V cavities, it was reported that the lowest microleakage scores were observed at the non-microabraded occlusal margins [63]. Given this extant research, microabrasion was not included in the current study, as it is not superior to the conventional method.

Therefore, the present study was conducted to evaluate the incidence of microleakage after preparation of the fissures using the different preparation techniques of conventional methods, enameloplasty and fissurotomy, followed by the application of a flowable composite.

Some studies have reported a need to perform enameloplasty to increase retention, because there is a nonprismatic enamel at the base of the fissures [64, 65]. It has been reported that fissure sealants placed on the teeth without enameloplasty failed to penetrate to the base of the fissure and were noncompliant [15]; in contrast, pit and fissure sealants placed on the teeth following enameloplasty were reported to achieve better retention than conventional ones [66]. In a study comparing the performance of fissure sealant materials with and without prior enameloplasty, flowable composite showed the best performance in terms of penetration and microleakage, and enameloplasty increased the penetration and impermeability of the sealants [67]. However, in the present study, enameloplasty was not superior to the other two methods in terms of microleakage.

Another surface preparation technique is the fissurotomy technique, in which pits and fissures are enlarged with a fissurotomy bur to create a greater surface area for the penetration of the sealant. It has been suggested that the use of a fissurotomy bur in conjunction with acid etching substantially increases the retention of the pit and fissure sealant [68]. In a study comparing acid etching alone, acid etching in conjunction with pumice prophylaxis, and fissurotomy in conjunction with acid etching, it was demonstrated that fissurotomy in conjunction with acid etching substantially decreased the incidence of microleakage compared to acid etching alone [69]. However, the present study found no significant difference between acid etching alone and fissurotomy in conjunction with acid etching.

Singh *et al.* [16] conducted a study comparing the different techniques of enameloplasty, fissurotomy and air abrasion; although the lowest microleakage incidence was reported in the air abrasion group, the authors suggested the use of minimally invasive methods to reduce the incidence of microleakage and to prevent secondary caries. In another study comparing conventional techniques, enameloplasty and fissurotomy, Chaitra *et al.* [27] reported the mean microleakage rates of 1.75, 0.5 and 1.5, respectively. The lowest microleakage rate was observed in the enameloplasty group compared to the other groups. In contrast to those findings, the present study found no significant difference in microleakage between the three groups, with mean microleakage rates of 1.87, 1.88 and 1.81.

The methylene blue penetration test has proven to be a dependable method for assessing microleakage near pit and fissure sealants, as evidenced by numerous studies [41]. Additionally, this test offers notable advantages, such as affordability and user-friendliness. Furthermore, microscopic images enable the measurement of various parameters and facilitate the detailed examination of samples [70]. In a study comparing two different dyes (methylene blue and silver nitrate), the measurability of parameters is limited when solely relying on the evaluation of sealant material following the dissolution of dental hard tissue in the samples [71]. In contrast, utilizing methylene blue staining enables the assessment of additional parameters, such as fissure shape, void count and sealant penetration depth (as demonstrated in the current study) through sample sectioning with a microtome.

One of the main limitations of this *in vitro* study comparing microleakage scores is the absence of factors affecting the success of restorations in *in vivo* conditions, such as saliva, patient diet and patient oral hygiene practices. Future studies are needed that evaluate and compare microleakage scores after the application of flowable composite materials as a fissure sealant following different methods of preparation of the teeth. In addition, in future research, the sample size may be increased, and the study can be expanded through the use and evaluation of different flowable composite materials and surface preparation methods, in order to reach a more robust conclusion. Moreover, in this study, a comparison was made between the microleakage values of a designated flowable composite utilizing various tooth surface preparation methods; as such, no separate control group was established for the substance employed [27]. Instead, the conventional surface preparation technique was designated as the intended control group, enabling comprehensive comparisons across the other two surface preparation techniques, both with the control group and among themselves.

5. Conclusions

Colored composite materials are known to increase children's compliance with treatment, and the use of these materials is becoming widespread in pediatric dentistry. However, dentists are concerned about the lower resistance of this material compared to conventional composite materials. The present study reports that the incidence of microleakage when a colored flowable composite material was used as a pit and fissure sealant was within acceptable limits; thus, this study supports the clinical use of this material.

AVAILABILITY OF DATA AND MATERIALS

The data that support the findings of this study are available from the corresponding author—BKE, upon reasonable request.

AUTHOR CONTRIBUTIONS

BKE—contributed to the acquisition, analysis, interpretation of the data and wrote the draft; MK—contributed to the statistical analysis of gathered data and contributed to acquisition of data. BKE and MK—contributed to the final version of the manuscript.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Ethical approval was obtained from the Research Ethical Committee at the University of Health Sciences, Hamidiye Faculty of Dental Medicine (Approval no. 21/69). The informed consent process was completed for each participant.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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