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Research article

Microleakage, microgap, and shear bond strength of an infiltrant for pit and fissure sealing

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ABSTRACT

Objectives: This study aimed to evaluate the potential clinical application of an infiltrant with different etchants as pit and fissure sealants and to compare them with a conventional resin-based sealant.

Materials and methods: Seventy-five molars were randomly divided into three groups (n = 25): phosphoric acid etchant + conventional resin-based sealant (Group A); 15% hydrochloric acid etchant + infiltrant (Group B); phosphoric acid etchant + infiltrant (Group C). Fifteen teeth in each group were subjected to pit and fissure sealing procedures. After 500 thermocycling and methylene blue dye penetration, ten specimens were sectioned and the percentages of dye penetration were measured under a stereomicroscope. Another five teeth in each group were sectioned and the microgaps between materials and enamel surface were measured using electron microscope scanning. Ten teeth in each group were used to measure shear bond strength and the failure mode was analyzed.

Results: The results showed that infiltrant exhibited significantly less microleakage and microgap than resin-based sealant, no matter which echant was used. Although there was no significant difference betweern the three groups, infiltrant applied with 15% hydrochloric acid etching showed higher shear bond strength than resin-based sealant etching with 35% phosphoric acid.

Conclusions: The infiltrant has significant advantages in reducing the degree of microleakage and microgap. Moreover, the infiltrant could achieve the same bonding strength as conventional resin-based sealant. Although, manufacturers do not currently recommend the infiltrant for fissure sealing, the potential clinical application would be an off-label use.

Clinical relevance This report provides a theoretical basis for the potential clinical application of the infiltrant as a pit and fissure sealant, and provides a new perspective for selecting pit and fissure sealants.

1. Introduction

According to the Global Burden of Disease Study, 2.4 billion people around the world are affected by permanent dental caries [1]. There are some dental cusps and ridges on the occlusal surface of molars, which play a very important role in the process of eating and

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chewing. Pit and fissure refer to some inadequately calcified concave part and confluence of three or more developmental grooves. It has been reported in the literature to classify pit and fissure based on morphology as follows: V, U, I, IK, and inverted Y [2]. Owing to their anatomical structure, pits and fissures can easily retain cariogenic food residues and bacteria, which cause irreversible chronic and progressive destruction of hard dental tissue. Therefore, the occlusal surface is frequently affected by caries from the beginning of tooth eruption [3]. Previous methods for preventing **pit and fissure caries** included sealing with zinc phosphate cement, prophylactic restorations, and chemical treatment with silver nitrate. However, these methods have been replaced by superior sealing materials, including resin and glass ionomer cements [4,5]. Many studies have verified that **pit and fissure sealing (PFS)** can effectively prevent dental caries [6,7]. For permanent molars, PFS is recommended by the American Dental Association (ADA) [8].

Microleakage is an important factor in evaluating the sealing effect of the sealing material, and the smallest possible microleakage is crucial for the long-term efficacy of PFS [9]. Shear bonding strength (SBS) is commonly used to evaluate the bonding strength between the sealing material and enamel surfaces; a significant correlation has been found between SBS and 5-year retention [9]. Thus, SBS and microleakage testing experiments are often performed to evaluate the properties of PFS materials [10].

Resin-based sealants are the most commonly used sealing materials [11,12]. The composition of conventional resin-based sealant is resin matrix, diluent, initiator and some auxiliary agents (solvent, filler, fluoride, coating). The conventional sealer attaches to the micro-retentive enamel after etching with 35% phosphoric acid and hardens in tag-like projections, attaching to the tooth structure according to the micromechanical retention principle [13]. While resin-based sealants are effectively protect against caries, they also present some disadvantages. For example, the polymerization shrinkage of resin-based sealants can potentially result in a microgap between the enamel and the sealant, which allows saliva, cariogenic bacteria, and food residues to penetrate the microgap, thus leading to microleakage. In the long term, microleakage may decrease the retention rate of the sealing materials and even lead to caries under the sealant barrier [14,15]. Therefore, preventing microleakage is crucial to the long-term efficacy of PFS [16].

Recently, a resin infiltrant was developed to infiltrate the pores of the caries lesion with a high penetration coefficient and low viscosity, forming a resin-porous hydroxyapatite complex [17,18]. The main components of infiltrant are trivinyl - glycol - dimethacrylate resin, bisphenol A glycerol dimethacrylate, camphor, ethyl benzoate and solvent ethanol. It can also block the spread of bacteria and acids, effectively preventing caries development [19,20]. In addition, the infiltrant can conceal spot lesions after orthodontic treatment [19,21]. One clinical study demonstrated the successful correction of post-orthodontic lesions with infiltration treatment [22]. In contrast to other sealants, the infiltrant can form a resin-porous hydroxyapatite complex as a protective barrier inside the tooth [23]. Infiltrant is not recommended for fissure sealing by the manufacturer so far probably due to time consuming and high cost. However given its ability to better penetrate the enamel and form a protective barrier inside the tooth, we tried to explore the potential clinical application, which would be an off-label use. According to the recommendation by clinicians, 15% hydrochloric acid etching is required before infiltrant application to remove the superficial enamel layer from the white spot lesion. However, it is not yet known whether the conventional 35% phosphoric acid can be used for etching prior to infiltrant application in PFS.

The etching pattern might be quite similar, but acid etching before fissure sealing will remove the outer prismless enamel layer, while hydrochloric acid etching before caries infiltration has to remove the superficial enamel layer from the white spot lesion [24]. Therefore, the last procedure needs a "stronger" acid. In fissure caries lesions, infiltrant penetration after etching with 35% phosphoric acid was not as well as resin infiltration after etching with 15% hydrochloric acid [23], however, 35% phosphoric acid has the advantage of short operating time and high safety. Currently, no comparative trials have been performed using 35% phosphoric acid etching versus 15% hydrochloric acid etching followed by resin infiltration for PFS.

In the present work, we compared the microleakage, microgap, and SBS of 35% phosphoric acid or 15% hydrochloric acid etched within infiltrant for PFS and compared it with conventional resin-based sealant. The study aimed to explore the potential clinical application of infiltrant as a pit and fissure sealant.

2. Material and methods

The institutional review board of the hospital granted ethical approval to carry out the study and the participant's written consent was obtained before tooth extraction. The ethical approval date is Aug 27, 2021.

2.1. Sample selection

A total of 75 **healthy third molars** free of caries, enamel hypoplasia, and fillings were evaluated. There were deep pits and fissures on the occlusal surface (ICDAS code 0) [25], which met the indications for PFS. After extraction, the teeth were temporarily preserved in 75% ethanol (Macklin, Shanghai, China) for 24 h to kill bacteria and viruses. All teeth were cleaned of periodontal tissues and stored in a 0.1% thymol solution for 24 h [26]. All teeth were embedded (1-mm-below) in epoxy resin (CER 1000, Weiyee, China), and then the crowns were cleaned with a bristle brush and rinsed with water spray. All teeth were randomly divided into three groups (n = 25) according to the following procedure:

Group A: 35% phosphoric acid (Gluma Etching, Heraeus, Germany) + conventional resin-based sealant (Helioseal F, Ivoclar Vivadent, Liechtenstein).

Group B: 15% hydrochloric acid + infiltrant (Icon, DMG, Germany).

Group C: Soft-etch with 35% phosphoric acid (Gluma Etching, Heraeus, Germany) + infiltrant.

2.2. Specimen preparation for microleakage

Ten teeth from each group were randomly selected for microleakage and microgap evaluation.

2.2.1. Group A

A 35% phosphoric acid etchant gel was applied to the occlusal surfaces for 30 s, reaching 2/3 of the tip slope, then rinsed with water spray for 20 s. The samples were dried with air spray for 10–15 s, and a chalky-white enamel surface was obtained. A conventional resin-based sealant was applied to the occlusal surfaces and then light-cured with an LED curing unit (Kerr Demi Plus LED, Kavo, Germany) in standard power mode (1100 mW/cm^2) for 20 s [27].

2.2.2. Group B

Following the manufacturer's instructions, the surface was treated with 15% HCl gel (Icon, DMG, Germany) for 2 min and rinsed thoroughly for 30 s. Consequently, the fissures were dried with absolute ethyl alcohol for 30 s and air-dried for another 30 s. The infiltrant was applied evenly on the pit and fissures and protected from light for 3 min before light curing. The process was repeated (after penetrating for 1 min) until any residual porosity was filled.

2.2.3. Group C

A 35% phosphoric acid etchant was used as described in Group A, followed by the infiltrant application after drying with absolute ethanol described in Group B.

2.3. Specimen aging testing

Based on previous studies, a thermocycling bath was used to simulate clinical aging of materials [26]. Briefly, thirty teeth were placed in 37 °C distilled water for 24 h and then followed by a thermocycling bath (TC-501 F, Weier, China) between 5 (±2)°C and 55 (±2)°C. The residence time of each temperature-controlled water tank was 30 s, and the conversion time was 5 s.

2.4. Evaluation of microleakage

Two layers of nail polish were applied to cover the tooth crown after marking it 1 mm away from the edge of the sealer to prevent the methylene blue dye from penetrating the tooth surface cracks. After the nail polish was dried, we soaked the samples in a 2% methylene blue solution at 37 °C for 24 h, followed by rinsing with running water to remove the remaining dye. All samples were buccolingually sectioned with a precision saw (ISOMET HS PRO, Buehler, USA) into at least 2-mm-thick slices. Each slice was analyzed twice by two researchers who were blind to the design grouping under a stereomicroscope (SteREO Discovery, V20 Zeiss, Germany) at a 50 × magnification, with an interval of one week. The mathematical formula for microleakage measurement is as follows: microleakage = blue length/total length. Blue length refers to the length of methylene blue dye leakage, shown as a blue line. The total length refers to the total length of the edge line at the interface between the material and the enamel. The average of the four measurements was taken as the final result. If significant dye penetrated the enamel and/or dentine cracks or along the cracks of the sealant, it was not regarded as microleakage.

2.5. Measurement of the microgap

Five teeth in each group were subjected to PFS, as described above, without thermocycling bath aging. The roots of all samples were cut along the cement-oenamel junction, and the crowns were buccolingually cut into two fragments with a high-speed precision saw, producing 30 sections in total, all of which were gold-sputtered and inspected by SEM. The 2-mm-area at the center of the fissure was selected, and the widest width of the microgap between the sealant and the enamel surface was measured using a scanning electron microscope (5000 ×) by two researchers who were blind to the design grouping at different times (one week apart), and finally averaged the four measurements.

2.6. Shear bonding strength test

Ten teeth in each group were tested for shear bonding strength (SBS). The test method for SBS is based on the Ultradent method with appropriate modifications [28]. Surface preparation was necessary before the bonding procedure, and a two-step sequential planning process was suggested. First, P120 paper was used to grind the enamel surface until the bonding area was sufficient for placing a resin cylinder with a diameter of 2.38 mm. Then, P400 paper was used to grind the enamel until the surface was smooth upon visual inspection. After the cleaning and etching described above, a 2.38 mm × 5 mm rubber cylindrical mold was placed on the enamel plane. A fluid gingival barrier resin was applied in case of possible leaks, followed by sealant filling and light-curing to prevent the outflow of materials from underneath the mold. Subsequently, the teeth were stored in humid conditions at 37 °C for 24 h, and the universal testing machine (5967, Instron, USA) was used for the SBS test according to a previous study. The base of the specimens was fixed with a specific fixture, and a shallow concave shear blade was installed on the universal testing machine (5967, Instron, USA) and placed over the cylinder. The shear blade applied force at a constant speed to the cylinder and recorded the maximum force when the cylinder was cracked. The ratio of the maximum force to the bonding area was calculated as the SBS of the material.

2.7. Analysis of failure mode

All specimens and failure modes were analyzed under a stereomicroscope at a magnification of 50 × . Failure modes were classified into four types: (1) enamel failure; (2) adhesive failure; (3) cohesive failure within the material; or (4) mixed failure (adhesive and cohesive within the material). The analyst was blind to the experimental grouping and the failure mode was noted according to the randomized number of specimens.

2.8. Statistical analysis

Statistical analyses were conducted using SPSS 20.0 (IBM) software. All the data were represented as mean ± standard deviation (SD). Normality test and homogeneity test of variance were first conducted. If the data conformed to normal distribution and did not conform to homogeneity of variance, welch test was used for population mean comparison and Dunnett T3 test was used for pair-to-pair comparison between groups; If the data does not conform to normal distribution and homogeneity of variance, the Mann-Whitney *U* test was used for pair-to-pair comparison between groups. A p-value of less than 0.05 was considered statistically significant.

3. Results

3.1. Infiltrant exhibits less microleakage

Group A showed the highest microleakage, significantly higher than in Groups B and C (Fig. 1 and Table 1). Microleakage was not significantly different between Groups B and C ($p > 0.05$). Images obtained under the stereoscopic microscope are shown in Fig. 2. Obvious microleakage was observed in group A, as shown by the dye penetrating from the edge of the material to the middle section of the fissure. Conversely, no obvious dye solution penetration was observed in groups B and C, and the interface between the material and enamel did not show blue staining (see Fig. 2).

3.2. The infiltrant has significant advantages in reducing the degree of microgap

The microgap widths in Groups A, B, and C were 2.19 + 2.18 μm, 0.65 + 0.89 μm, and 0.62 + 0.75 μm, respectively (Table 2). The microgaps in Group A were significantly larger than those in Groups B and C ($p < 0.05$), while the microgaps in Group B were slightly larger than those in Group C, but this was not statistically significant ($p > 0.05$). There was a uniform linear gap between the resin-based sealant and enamel in group A, while the results of electron microscopy in groups B and C showed almost no microgap between the infiltrant and enamel, regardless of which etchant wash used: 15% hydrochloric acid for 2 min or 35% phosphoric acid for 30 s (Fig. 3) (see Fig. 4).

3.3. Infiltrant gives the same adhesive strength as conventional fissure sealing materials

Group B showed the highest SBS, while Group C showed the lowest (Table 3 and Fig. 5), but the difference between the three groups was not statistically significant ($p > 0.05$). The results of the failure mode analyses are presented in Table 4. The most common failure type was the adhesive failure (70.00–80.00%) in all three groups, and only Group A exhibited enamel failure (20.00%).

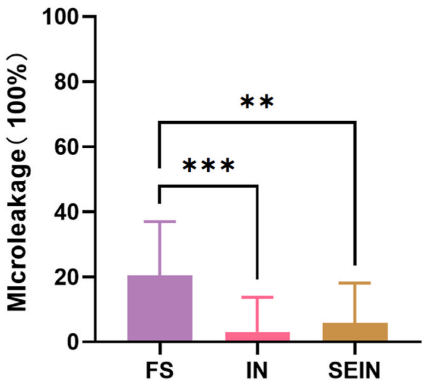

Fig. 1. Statistical results of microleakage. **means $p < 0.01$, ***means $p < 0.001$ Group A (FS) showed higher microleakage than Group B (IN) and Group C(SEIN). The difference between Group B (IN) and Group C (SEIN) is not statistically significant. * represents statistically significant differences between groups.

Table 1
Microleakage of the tested materials after 500 × thermocycling.

Category	Group A phosphoric acid/conventional fissure resin-based sealant (FS)	Group B hydrochloric acid/ infiltrant (IN)	Group C Soft-etch-phosphoric acid/ infiltrant (SEIN)
Number of teeth (N)	10	10	10
Number of all available specimens	18	18	18
Surfaces with no dye penetration(N)	1	16	14
Surfaces with dye penetration N (%)	17 (94.44%)	2 (11.11%)	4 (22.22%)
Mean microleakage (SD)	20.40% 16.60%	3.07% 10.65%	5.89% 12.21%
Minimum	0	0	0
Maximum	58.67%	44.54%	42.56%

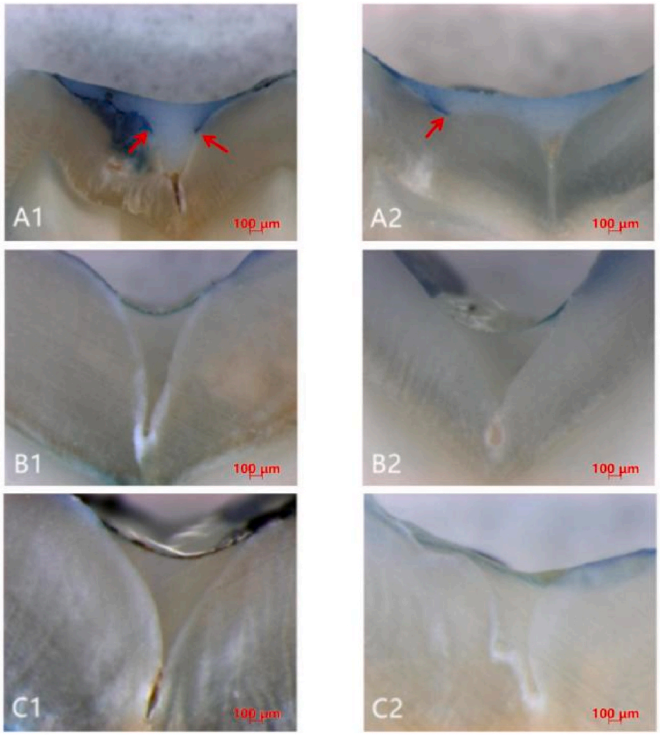

Fig. 2. Examples of microleakage results. Images obtained from the stereomicroscope at 50 × magnification are shown in Fig. 2. A1, A2: Group A (FS): Dye penetrated from the edge of the materials to the middle of the fissures, marked with a red arrow. B1, B2: Group B (IN): No obvious dye penetration was observed. C1, C2: Group C (SEIN): No obvious dye penetration was observed. The red arrow points to the dye leak

Table 2
SEM analysis.

Gap width μm)	N	Min	Max	Mean value	SD
Group A	10	0.41	6.17	2.19	2.18
Group B	10	0	2.55	0.65	0.89
Group C	10	0	2.08	0.62	0.75

4. Discussion

The study analyzed the microleakage, microgap, and SBS of infiltrants when applied with different etchants and resin-based sealants for PFS. The edge and internal suitability of restorative materials are assessed by the microleakage test, and dye penetration is the most common and convenient method. However, there is no internationally accepted standard evaluation method for the

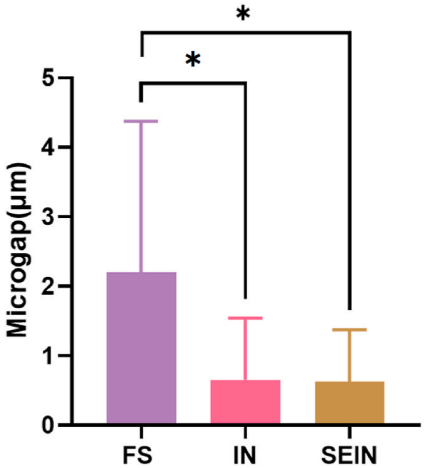

Fig. 3. Statistical results of the microgap. *means $p < 0.05$. The mean microgap of Group A (FS) is over 2 μm, which was the widest among all groups, while that of Group B (IN) and Group C (SEIN) is less than 1 μm. The difference between Group B (IN) and Group C (SEIN) is not statistically significant. * represents statistically significant differences between groups.

Table 3
Shear bond strength of groups.

Shear bond strength (MPa)	N	Min (Mpa)	Max (Mpa)	Mean value (Mpa)	SD (Mpa)
Group A	10	5.20	24.39	12.75	5.73
Group B	10	10.10	20.93	14.68	3.85
Group C	10	7.55	15.89	11.40	2.79

microleakage of PFS. Numerical scales, dichotomous scales, and percentage scales can be used to evaluate microleakage, and German-Cecilia demonstrated that percentage scales are more accurate than other scales [26]. Therefore, in the present study, microleakage was defined as the percentage of penetration depth of the methylene blue dye along the microgap between the sealant and the enamel surface. Taken together, the present findings confirmed that the microleakage of infiltrant was much lower than that of conventional resin-based sealant, regardless of 15% hydrochloric acid or 35% phosphoric acid etching. The 15% hydrochloric acid/infiltrant group performed best, and the leakage of dye solution in this was very rare. Amani et al. found that the infiltrant showed lower microleakage when sealing pits and fissures than the resin-based sealant [20], consistent with our results. The infiltrant with a high penetration coefficient [17] could penetrate the pores on the enamel surface via capillary action and form a resin-porous hydroxyapatite complex through which even small molecules of methylene blue dye were difficult to pass. This indicates that the infiltrant possesses a good marginal sealing property.

We found that a gap frequently occurred between the materials and enamel on the edge of the fissures on the 50 × SEM images. This may be due to polymerization shrinkage caused by shear stress, which results in cracks at the top of the fissure. At the bottom, fissures are difficult to fill completely due to the narrow scale, which makes it difficult for materials to penetrate fully. Therefore, to avoid the influence of polymerization shrinkage caused by shear stress and fissure morphology, a 2 mm area at the center of the fissure was selected for observation, and the width of the microgap between the sealing materials and the enamel was measured under a 5000 × SEM. The conventional resin-based sealant microgap was the largest in all groups. Different etchants did not affect on the microgap when applied with an infiltrant, consistent with the microleakage results. It is easier for small molecules of methylene blue dye to pass through with a wider microgap, and the higher the microleakage.

It has been suggested that etching with phosphoric acid results in a tremendous increase in the surface area of the enamel and the exposure of the organic framework of the enamel, which serves as a bonding network [29]. The volume of the pores on the enamel surface influences to a large extent the depth of resin penetration [30]. Acid etching can remove part of the surface layer of the enamel and increase the surface porosity, which facilitates deeper infiltration of the bonding material into the enamel and form the surface barrier [31]. The increased porosity of the outer enamel layer provides a retentive surface [32]. Meyer-Lueckel et al. found that treating enamel with a 15% hydrochloric acid etching agent for 90–120 s can almost completely remove the lesion surface. Therefore, hydrochloric acid etching is recommended to facilitate subsequent application of resin infiltration in natural enamel lesions [30]. For the healthy enamel, the results of the current study showed no significant difference in the microleakage, microgap, and SBS when using 15% hydrochloric acid for 2 min or 35% phosphoric acid for 30 s, indicating that it is feasible to use an infiltrant with 35% phosphoric acid etching on the healthy enamel.

Several methods can be used to measure the enamel bonding strength. Depending on the size of the bonding area, the bonding strength can be analyzed using a macro or micro test setup. When the bonding area is larger than 3 mm^2, the macro-bond strength can be measured in "shear," "tensile," or using a "push-out" protocol [24]. The SBS test is the most commonly used method, accounting for

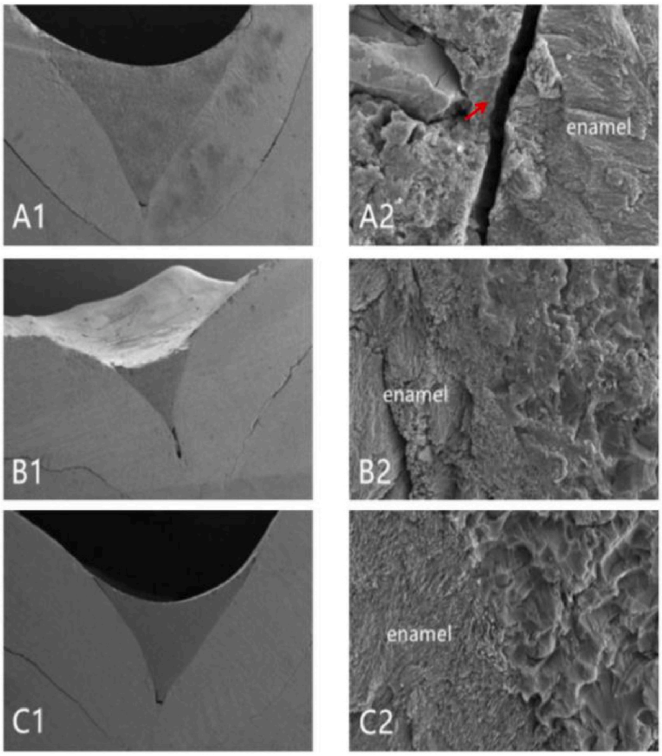

Fig. 4. Microphotographs of the sections. A1, A2: A SEM 50 × and 5000 × microphotograph of Group A (FS), respectively; fissure is not completely filled by fissure sealant, identified by a red arrow. B1, B2: A SEM 50 × and 5000 × microphotograph of Group B (IN), respectively; no obvious microgap is observed. C1, C2: A SEM 50 × and 5000 × microphotograph of Group C (SEIN), respectively; no obvious microgap is observed. The red arrow indicates a microgap

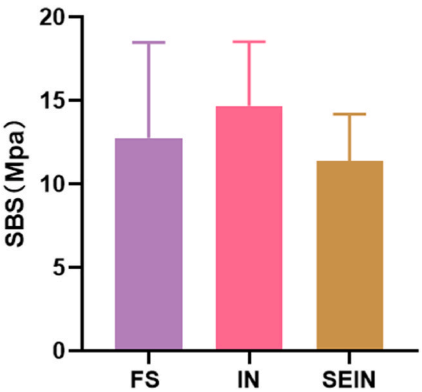

Fig. 5. Statistical results of SBS. The shear bond strength (SBS) of groups are all over 10 Mpa, and the difference among the three groups is not statistically significant ($p > 0.05$). The SBS of group B (IN) is slightly higher than that of group A (FS), while the SBS of group C (SEIN) was the lowest among the groups.

Table 4
Failure mode analysis of groups after measuring the shear bond strength.

Failure mode analysis (N/%)	Group A	Group B	Group C	Summation
Adhesive failure	7 (70.00%)	7 (70.00%)	8 (80.00%)	22 (73.33%)
Cohesive failure	–	–	–	–
Mixed failure	1 (10.00%)	3 (30.00%)	2 (20.00%)	6 (20.00%)
Enamel failure	2 (20.00%)	–	–	2 (6.67%)

approximately 26% of scientific research [33] owing to its simplicity. To date, the SBS of the infiltrant used for sealing pits and fissures has not been reported. The International Organization for Standardization (ISO) recommends a flat-shear blade for measuring the SBS rather than a wire loop. While prismatic enamel is required in the ISO standard, aprismatic enamel was used in the current investigation to simulate clinical PFS. Based on this consideration, it is challenging to pose a cylindrical plastic mold on the enamel surface without a gap. A fluid gingival barrier resin was applied in case of possible leaks to prevent the outflow of materials from underneath the mold,. According to the results of the SBS test, we cannot reject the hypothesis that all tested materials would perform equally. In this study, the differences in SBS among the three groups were not statistically significant, suggesting that the bond strength of the infiltrant was similar to that of the conventional resin-based sealant and different etchants did not have an effect.

Studies have found a strong correlation between failure mode and the average bond strength. In general, the higher the bond strength of the adhesive material, the higher the probability of cohesive failure. Kelly et al. stated that only a specimen with a fracture in the adhesive surface could accurately reflect the bond strength, and the other failure modes should not be counted [34]. In this study, the most common failure types were adhesive failure (70.00–80.00%), followed by mixed failure (10.00–30.00%), and the effective failure mode accounted for a high proportion. Therefore, the results of this study are credible.

This study does have some limitations. Firstly, a thermocycling bath was used to simulate clinical aging of materials, while the internal environment of the mouth is complex and changeable and the in vitro experiments failed to fully simulate the effects of organic solvents, physical friction, microorganisms, enzymes, and corrosive media in saliva on resin composites in the oral cavity. In addition, the abrasion resistance as well as the compressive strength of the PFS materials are also very important observations. Therefore, more clinical experimental studies are needed to investigate the long-term effects of different **PFS** materials in real oral environments.

5. Conclusion

The infiltrant has significant advantages in reducing the degree of microleakage and microgap. The infiltrant could achieve the same bonding strength as traditional pit and fissure sealants. When an infiltrant is used as a sealant, phosphoric acid etching can obtain a favorable marginal seal comparable to hydrochloric acid etching and consequently simplify the procedure. Altogether, this report provides a theoretical basis for the potential clinical application of the infiltrant as a pit and fissure sealant and provides a new perspective for selecting pit and fissure sealant.

5.1. Shortcomings and outlook

Although the manufactures does not currently recommend the infiltrant for fissure sealing, the potential clinical application of the infiltrant would be an off-label use. However, the high product costs and needed time for application may contra-indicate its clinical use for fissure sealing. Further in vivo studies are needed to confirm its clinical efficacy.

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Author contribution statement

Yueshan Zhou: Conceived and designed the experiments; Performed the experiments; Wrote the paper.
Xiuhong Huang: Contributed reagents, materials, analysis tools or data.
Linmei Wu; Yi Huang: Analyzed and interpreted the data.
Yihao Liang: Performed the experiments.
Shaohong Huang: Conceived and designed the experiments.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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