INTRODUCTION

Caries is diagnosed frequently in pits and fissures on permanent molars due to their anatomical characteristics and plaque-retentive nature. Therefore, preventive pit and fissure sealing is clinically recommended, particularly in young patients with caries activity, and have been assessed as caries-preventive procedures. Various sealing materials/procedures have been proposed for clinical use over the decades. As a measure of performance, the retention rates of resin-based materials are superior to those of other sealants such as glass-ionomer cements. Clinical usage requires simple and less-time consuming procedures, particularly in children. The application of self-etching adhesives instead of acid etching might simplify the clinical workflow, reduce application times and achieve acceptable cooperation from children. Most recently, a new product (BeautiSealant, Shofu, Kyoto, Japan) featuring reduced working time and fluoride-releasing and pH-buffering properties was released on the dental market. Stringent testing of new materials is imperative before formulating clinical recommendations. Shear bond strength (SBS) and microleakage assessment are frequently used to evaluate sealant materials in the laboratory. No study has evaluated the adhesion properties of this new material to predict the survival of the material clinically. Therefore, this study aimed to study the SBS and microleakage of the new sealant in relation to acid etching. The null hypothesis of this study was that there is no difference in SBS and microleakage between the tested material and the conventional pit and fissure sealant, which served as a control group.

MATERIALS AND METHODS

For this study, 60 healthy, caries-free extracted human third molars were used. The teeth were free of development disorders, fillings, and fissure sealants and showed complete root development. After extraction, the teeth were stored in sodium azide solution (0.2%). Before using the teeth for this study, all teeth were cleaned of gross debris and air-dried. All roots were sectioned off 1 mm apical to the cemento-enamel junction, and the crowns were sectioned on 4 surfaces (mesial, distal, lingual, and buccal) with a diamond disc (Dental Diamond Disc, H 340-F-300, HORICO, Berlin, Germany), resulting in 240 tooth surfaces. Specimens were randomly assigned to each group (n=120) and stored in distilled water. All tooth surfaces were embedded in cold-curing methyl methacrylate resin (Technovit 4004, Heraeus Kulzer, Wehrheim, Germany). Each tooth surface was strictly aligned horizontally in the embedding material. All specimens were numbered according to the randomization table. After embedding, all tooth surfaces were cleaned and rinsed with water spray. Only unprepared specimens (prismless enamel) were used in this study to simulate the clinical situation of fissure sealing (Fig. 1).

Experimental group (EG) — Self-etch adhesive prior sealant application

The in vitro application of the sealant material strictly followed the clinical recommendations of the manufacturer. This self-etching/self-adhesive sealant, which served as a control group.
Fig. 1 An overview of the investigated sealants for the applied methods.

(BeautiSealant Paste, Shofu, LOT 021301) obviates additional acid etching. In this case, the tooth surface was rinsed with water spray and dried with water- and oil-free air. Care was taken to ensure that the enamel surface was not over-dried. The self-etching adhesive (BeautiSealant Primer, Shofu) was brushed onto the tooth surface with an applicator tip and allowed to set for 5 s. Subsequently, the adhesive was dried thoroughly into a thin, smooth and glossy film using a gentle stream of air. In the next step, a cylindrical plastic mould (Button Mould Insert, ISO 29022, Ultradent Products, South Jordan, UT, USA) in a bonding clamp (ISO 29022, Ultradent Products) was placed gap-free on a flat area of the tooth surface, and the fissure sealant was applied onto the surface. To avoid outflow of the fissure sealing material, a light-cured resin barrier (OpalDam, Ultradent Products) was applied at the outside of the plastic mould before applying the material. Then, the fissure sealant was applied in the mould and light-cured for 20 s with a polymerization light (Bluephase, 1,200 mW/cm², wavelength 385–515 nm, Ivoclar Vivadent, Schaan, Liechtenstein). The plastic mould and resin barrier were carefully removed. The final sealant cylinder on the tooth surface was 2.38 mm in diameter, equal to the standardized diameter of the cylindrical plastic mould required by ISO 29022.

Control group (CG) — Conventional fissure sealing with 30-s acid etching
The tooth surface was initially rinsed with water spray and air-dried with water- and oil-free air. Next, the enamel surface was etched with 37% phosphoric acid gel (Total Etch, Ivoclar Vivadent) for 30 s. The tooth surface was rinsed with water spray and dried with pressured air for 5 s until a chalky-white enamel surface was visible. The fissure sealing material (Helioseal F, Ivoclar Vivadent, LOT S03724) was applied through the plastic mould in a layer of 2.38 mm and light-cured as indicated above, resulting in a sealant cylinder on the tooth surface.

All specimens were checked for proper configuration, and specimens with bubbles and/or outflow sealing material were discarded. There were no quality deficits after treatment and no initial loss of sealant cylinders prior to SBS testing.

Alteration of the specimens
Each group was divided into 3 subgroups (n=40), which were altered using different protocols: a) 1-day storage in distilled water at 37°C in a thermal oven (Jouan EU3, INNOVENS Ovens, ThermoFisher Scientific, Waltham, MA, USA); b) 3 months storage in distilled water at 37°C in a thermal oven; and c) 1-day storage in distilled water at 37°C followed by a thermocycling bath (Haake W15, Thermo Haake, Karlsruhe, Germany) between 5(±2) and 55(±2)°C for 5,000 cycles with a dwell time of 30 s and a transfer time of 5 s.

SBS
A standardized Ultradent-method (Notched-edge SBS test, ISO 29022) was employed to test the SBS. After specimen storage, the SBS test was performed in a universal testing machine (MCE 2000ST, Quicktest Prüfpartner, Langenfeld, Germany) at a crosshead speed of 1 mm/min. The specimens were aligned in a metal sample holder (Test base clamp, ISO 29022, Ultradent Products) with the occlusal tooth surface facing down (“crown down”). The notched-edge shear fixture (Notched-edge crosshead assembly, ISO 29022, Ultradent Products) with the semi-circular moulded shear blade (Notched-edge shear blade, ISO 29022, Ultradent Products) mounted to the universal testing machine and placed over the sealant cylinder on the aligned specimen. The semi-circular moulded shear blade was positioned exactly over the sealant cylinder and force-fitted without premature contact to ensure that the load was applied directly to the sealant cylinder. A constant crosshead speed of 1 mm/min was applied until the material failed. The maximum force (N) until failure was recorded. The SBS was calculated based on the bonding area of the fissure sealant on the tooth surface and is expressed in MPa.
**Failure mode analysis**

The failure modes of all specimens were examined using a stereomicroscope (Stemi SV11, Carl Zeiss, Jena, Germany) with a 20-fold magnification. Failures were classified as 1) adhesive failure (complete debonding of material); 2) cohesive failure within the material; 3) mixed failure (partial adhesive and cohesive within material); and 4) enamel failure. The operator was blinded to the tested group, and the failure mode tests were performed and scored based on the randomization number of the specimen. The data management personnel reorganized the values into the investigated groups based on their identification numbers.

**Microleakage**

Eight human third molars were assigned to each of the 2 groups (EG: 80 sections; CG: 104 sections). All teeth were stored and cleaned as previously described. Each tooth was taken as a whole, and fissure sealing on the prismatic enamel of the occlusal fissure pattern was performed in strict accordance with the manufacturer's instructions (see above). All specimens were stored in distilled water at 37°C for 24 h in a thermal oven (Jouan EU3, INNOVENS Ovens, ThermoFisher Scientific). In the next step, the specimens were aged in a thermocycling bath (Haake W15, Thermo Haake) between 5(+2)°C and 55(±2)°C for 5,000 cycles, with a 30 s dwell time and a transfer time of 5 s. After thermocycling, the root surfaces were isolated with tacky wax (Boxing Wax Sticks, Kerr, Romulus, MI, USA). Afterwards, the entire tooth surface was covered with two layers of nail varnish, except the area within 1 mm of the fissure sealing. The varnish was applied to avoid dye penetration to other parts of the tooth. The specimens were then immersed in 0.5% basic fuchsin solution for 24 h at 37°C. All specimens were rinsed with water, and the roots were sectioned off 1 mm below the cemento-enamel junction with a diamond disc (Dental Diamond Disc, H 340-F-300, HORICO). The tooth crowns were then fully embedded in cold-curing methacrylate resin (Technovit 4004, Heraeus Kulzer). This treatment resulted in a rectangular block of approximately 2.5×1.2×0.8 cm for each tooth. The blocks were fixed in a sectioning saw (Isomet Low Speed Saw, Buehler, Lake Bluff, IL, USA) with a diamond blade (Diamond Blade, Leco, St. Joseph, MI, USA), and the crowns were sectioned (in a buccolingual direction) into at least 5 slices, each with a thickness of 1 mm. The front and back of each slice were inspected, resulting at least 10 available section sides per tooth. The side analysis was performed using a stereomicroscope (Stemi SV11, Carl Zeiss) with a 20-fold magnification. Every side was photographed with a digital single-lens reflex camera. The following picture analysis methodically separated all sides without dye penetration and then collected all section sides with dye penetration. Additionally, quality losses, such as dye penetration at sealant fractures, detachment of sealant, and defects of the fissure sealant, were recorded. If dye penetration was present, each side was quantitatively measured in relation to the total length of the interface between the enamel and the sealant. All measurements were performed with the imaging software ImageJ (Version 1.47, Wayne Rasband, National Institutes of Health, Bethesda, MD, USA). The percentage of microleakage was calculated. Microleakage was ruled out for dye penetration through enamel, dentine or fissure sealant cracks or along the cemento-enamel junction.

**Statistical analyses**

A formal sample size calculation was performed using 80% power, a 95% confidence level and a two-tailed significance level of 5%. The least expected mean difference in the SBS between the two groups was set at 3 MPa with a variance of 20 and 25 for the two groups, respectively. This resulted in a sample size of 40 specimens per group (10 pieces each from mesial, buccal, lingual and distal). Furthermore, to avoid the influence of some teeth on the results, we randomized the pieces such that no piece of a tooth was repeated more than once in a group. The descriptive and explorative data analyses were performed using R, version 3.3.1 (R Core Team, 2016). Descriptive statistics for SBS and microleakage were calculated and presented as the mean and standard deviation or as percentages, respectively. Because the data were not normally distributed, pairwise comparisons with respect to the material and the technique employed were performed using the Mann-Whitney U-Test. Furthermore, multiple linear regression was performed to study the influence of different factors such as material (EG and CG), alteration technique (1-day water storage, 3-month water storage and 5,000× thermocycling) and tooth surface (mesial, buccal, lingual and distal) on the SBS. The adjusted estimates, their standard errors and significance values were calculated. A two-tailed significance level of 0.05 and a 95% confidence level were used for all analyses. Another linear regression model was performed including an interaction term between the material and alteration technique; but the results were not significant and thus are not reported.

**RESULTS**

The results of the SBS test are shown in Table 1. The SBS decreased slightly in both groups after 3-month water storage and significantly after 5,000 cycles of thermocycling. The SBS in the EG was significantly lower and ranged between 3.2 and 4.6 MPa, whereas the SBS in the CG ranged from 15.6 to 19.1 MPa. Further analysis using multiple linear regression models revealed significant influences of both factors on the SBS (Table 2). The mean SBS estimate was 3.9 MPa for the intercept and 13.6 MPa for the CG. The linear model indicated that 3-month water storage had no significant deteriorating effect compared to that of 1-day water storage. However, 5,000 cycles of thermocycling significantly reduced the SBS by −2.5 MPa on average. The surface was a significant factor but had minimal influence on the SBS. The evaluation of the failure mode analysis is presented in Table 3. Adhesive failure...
Table 1  Shear bond strength for the tested sealant materials (EG and CG)

| Tested sealant                          | Shear Bond Strength in MPa Mean (standard deviation) | Min–Max                  |
|--------------------------------------|-----------------------------------------------------|--------------------------|
|                                      | 1-day water storage                                | 3-month water storage    | 5,000 cycles of thermocycling |
|                                      | Mean (standard deviation)                           |                          |                          |
| Fissure sealing with self-etch adhesive (EG) | 4.6 (1.6)\textsuperscript{1,a}                    | 4.4 (2.2)\textsuperscript{b} | 3.2 (2.0)\textsuperscript{1,c} |
|                                      | 2.0–8.4                                              | 0.2–11.8                 | 0.1–7.4                  |
| Fissure sealing with 30 s acid etching (CG) | 19.1 (6.2)\textsuperscript{1,a}                  | 18.2 (7.5)\textsuperscript{b} | 15.6 (4.4)\textsuperscript{1,c} |
|                                      | 8.8–35.3                                             | 5.1–30.3                 | 6.1–22.3                 |

\textsuperscript{1} indicates statistically significant difference between 1-day water storage and 5,000×thermocycling (\(p<0.05\))

\(a, b, c\) indicates statistically significant difference between the linked groups (\(p<0.05\))

Table 2  Multiple linear regression results for the influence of material, alteration and surface on the shear bond strength of the fissure sealants

| Factor      | Factor level | Estimate for SBS in MPa | Standard error | \(p\)  |
|-------------|--------------|--------------------------|----------------|--------|
| Reference   | —            | 3.9                      | 0.8            | —      |
| Material    | CG           | 13.6                     | 0.6            | <0.001* |
| Alteration  | 3-month water storage | —                     | —              | 0.002* |
|             | 5,000 cycles of thermocycling | —                     | —              | 0.002* |
| Surface     | Buccal       | 0.6                      | 0.8            | 0.01*  |
|             | Lingual      | 2.6                      | 0.8            | 0.01*  |
|             | Distal       | 1.5                      | 0.8            | 0.01*  |

The estimate at the reference level corresponds to the EG under 1-day water storage on the mesial surface

\* indicates significance under multiple linear regression analysis (\(p<0.05\))

Table 3  Failure mode analysis of the tested sealant materials (EG and CG) following the shear bond strength measurements

| Tested sealant                          | Type of failure | Failure mode analysis N (%) |
|--------------------------------------|----------------|-----------------------------|
|                                      |                | 1-day water storage | 3-month water storage | 5,000 cycles of thermocycling |
| Fissure sealing with self-etch adhesive (EG) | Adhesive       | 40 (100)                 | 40 (100)                 | 40 (100)                 |
|                                      | Cohesive       | —                         | —                         | —                         |
|                                      | Mixed          | —                         | —                         | —                         |
|                                      | Enamel         | —                         | —                         | —                         |
| Fissure sealing with 30 s acid etching (CG) | Adhesive       | 31 (77.5)                | 32 (80.0)                | 38 (95.0)                |
|                                      | Cohesive       | —                         | —                         | —                         |
|                                      | Mixed          | 9 (22.5)                 | 7 (17.5)                 | 2 (5.0)                  |
|                                      | Enamel         | —                         | 1 (2.5)                  | —                         |

was the only cause of failure in the EG and the most predominant failure type in the CG, ranging between 77.5 and 95%, followed by mixed failures, which ranged between 5 and 22.5%.

Table 4 illustrates the results from the microleakage analyses. Although the observed number of sites with any quality loss was similar in the EG and the CG, the mean microleakage was significantly higher in the EG (12.8%) than that in the CG (1.1%). The highest amount of microleakage observed was 95.9% in the EG but 36.1% in the CG.
Table 4 Microleakage of the tested sealant materials (EG and CG) after 5,000 cycles of thermocycling

| Microleakage | Fissure sealing with self-etch adhesive (EG) | Fissure sealing with 30 s acid etching (CG) |
|--------------|--------------------------------------------|--------------------------------------------|
| Number of teeth, N | 8 | 8 |
| Number of available tooth specimens, N | 80 | 104 |
| Surfaces with no dye penetration, N | 58 | 83 |
| Surfaces with dye penetration, N | 22 | 19 |
| Surfaces with dye penetration at sealant fractures, N | 5 | 2 |
| Detachment of fissure sealant | — | — |
| Sites with any quality loss, N (%) | 27 (33.8%) | 21 (20.2%) |
| Microleakage, mean % (SD) | 12.8% (±26.8)* | 1.1% (±3.9)* |
| Range of microleakage, min–max | 0–95.9% | 0–36.1% |

* indicates statistically significant difference (p<0.05)

DISCUSSION

Based on our findings, the hypothesis that the tested sealant performs equally well is rejected. Within the limitations of this study, the performance of the newly developed self-etching fissure sealant in the SBS and microleakage tests was significantly inferior compared to that of conventional fissure sealing with 30 s of phosphoric acid etching. This result was consistent for different alteration procedures (Tables 1 and 2), and 5,000 cycles of thermocycling degraded the SBS most aggressively, by nearly −2.5 MPa. The SBS for the EG ranged from only 3.2 to 4.6 MPa, whereas in the CG, values were registered between 15.6 and 19.1 MPa. These findings are somewhat consistent with previously published studies of self-etching/self-adhesively bonded sealant materials. Several studies have reported results within the same order of magnitude or lower SBS values11-13). To further confirm the results, multiple linear regression was performed and yielded similar estimates for the two alteration methods (Table 2). Although these new self-etching/self-adhesive sealants have the potential to simplify the clinical workflow, the bonding ability under laboratory conditions suggests reduced longevity in clinical practice, particularly with this experimental material. Therefore, the primer component of the experimental sealant material might be replaced by conventional acid etching. An in vitro study of a similar material observed improved performance when the acid etching step was included prior to fissure sealant application10, but such a modification of the protocol was not assessed in the EG in the present study. Moreover, 100% of all failures in the EG were due to adhesive failures, which is an indicator of reduced adhesive performance.

Similar to the results of the SBS analysis, the microleakage results in the EG were inferior (12.8%); in contrast, in the CG, little dye infiltration was observed (1.1%). A similar trend has been reported in the literature, in which a few studies have observed microleakage for the self-etch adhesive sealants in a range closer to that of acid-etch sealants14,15). However, extremely high microleakage values greater than those observed in our study have also been reported16). Few studies have assessed its additional fluoride-releasing property6) and pH buffering mechanism7,8). Though these cariostatic properties were not measured in this study, its use in clinical settings has to be decided based on weighing both its physical and cariostatic properties.

The methodological strengths of our study include the use of an equal sample size of 40 specimens per group and randomization within a group to avoid repeating analysis of the same specimen. To simulate the clinical situation of teeth for fissure sealing, we used aprismatic enamel because ground prismatic surface would lead to a flat surface, which is ideal for bonding but does not reflect the conditions of fissure sealing. All groups were consistently subjected to three types of alterations, such as 1-day water storage, 3-month water storage and 5,000 cycles of thermocycling. With this approach, we tested the material under various types of stress exposures that might be encountered in the oral cavity. 1-day (frequently used control) and 3-month water storage helps to assess the hydrolytic degradation. Thermocycling was performed to assess the effect of thermal stress which is usually encountered in the oral cavity. 1-day (frequently used control) and 3-month water storage helps to assess the hydrolytic degradation. Thermocycling was performed to assess the effect of thermal stress which is usually encountered in the oral cavity. To test this physical characteristic in adhesive materials, a range of 250 to 2,500 thermocycling cycles is generally used9,17,18). Contrary to this “standard”, we followed the stringent method of subjecting the material to 5,000 cycles of thermocycling, which might indicate a long-term perspective on the longevity of the investigated sealant material. Further, we used a notch-edge shear technique, the most the recently recommended standardized method according to the International Organization for Standardization10.
CONCLUSION

New materials in the field of dentistry are essential, as are stringent quality control and testing of these materials. The tested self-etch adhesive pit and fissure sealant had roughly four-fold lower SBS, and the microleakage was ten-fold higher compared to that of the control group. Reduced SBS coupled with increased microleakage, as observed in this study, is an indicator that the performance of a fissure sealant is doubtful. Within the limitations of this study, the tested material failed in the physical characteristics to that of the control group, and thus its usage in clinical practice cannot be fully recommended.

REFERENCES

1) Kühnisch J, Galler M, Seitz M, Stich H, Lussi A, Hickel R, Kunzelmann KH, Bücher K. Irregularities below the enamel-dentin junction may predispose for fissure caries. J Dent Res 2012; 91: 1066-1070.
2) Hannigan A, O’Mullane DM, Barry D, Schäfer F, Roberts AJ. A caries susceptibility classification of tooth surfaces by survival time. Caries Res 2000; 34: 103-108.
3) Ahovuo-Saloranta A, Forss H, Hiiri A, Nordblad A, Mäkelä M. Pit and fissure sealants versus fluoride varnishes for preventing dental decay in the permanent teeth of children and adolescents. Cochrane Database Syst Rev 2016; 1: Cd003067.
4) Tinanoff N, Coll JA, Dhar V, Maas WR, Chhibber S, Zokaei L. Evidence-based update of pediatric dental restorative procedures: Preventive strategies. J Clin Pediatr Dent 2015; 39: 193-197.
5) Welbury R, Raadal M, Lygidakis NA. Eapd guidelines for the use of pit and fissure sealants. Eur J Paediatr Dent 2004; 5: 179-184.
6) Dionysopoulos D, Sfeikos T, Tolidis K. Fluoride release and recharging ability of new dental sealants. Eur Arch Paediatr Dent 2015; 17: 45-51.
7) Ushimura S, Nakamura K, Matuda Y, Minamikawa H, Abe S, Yawaka Y. Assessment of the inhibitory effects of fissure sealants on the demineralization of primary teeth using an automatic ph-cycling system. Dent Mater J 2016; 35: 316-324.
8) Kaga M, Kakuda S, Ida Y, Toshima H, Hashimoto M, Endo K, Sano H. Inhibition of enamel demineralization by buffering effect of s-prg filler-containing dental sealant. Eur J Oral Sci 2014; 122: 78-83.
9) Chiang ML, Birlbauer S, Lo YF, Pitchika V, Crispin A, Ilie N, Hickel R, Kühnisch J. Which factors influence the shear bond strength of sealant materials? J Adhes Dent 2016; 18: 397-404.
10) International Organisation for Standardisation. Iso 29022. Dentistry-adhesion-notched-edge shear bond strength test; 2013.
11) Wadonya RO, Yego C, Blatz MB, Mante F. Bond strength and microleakage of a new self-etch sealant. Quintessence Int 2009; 40: 559-563.
12) Dhillon JK, Pathak A. Comparative evaluation of shear bond strength of three pit and fissure sealants using conventional etch or self-etching primer. J Indian Soc Pedod Prev Dent 2012; 30: 288-292.
13) Margvelashvili M, Vichi A, Carrabba M, Goracci C, Ferrari M. Bond strength to unground enamel and sealing ability in pits and fissures of a new self-adhering flowable resin composite. J Clin Pediatr Dent 2013; 37: 397-402.
14) Javadinejad S, Razavi M, Birang R, Atefat M. In vitro study of microleakage of different techniques of surface preparation used in pits and fissures. Indian J Dent Res 2012; 23: 247-250.
15) Bassir L, Khanemhasjedi M, Nasr E, Kaviani A. An in vitro comparison of microleakage of two self-adhesive adhesives and the one-bottle adhesive used in pit and fissure sealant with or without saliva contamination. Indian J Dent Res 2012; 23: 806-810.
16) Schuldt C, Birlbauer S, Pitchika V, Crispin A, Hickel R, Ilie N, Kühnisch J. Shear bond strength and microleakage of a new self-etching/self-adhesive pit and fissure sealant. J Adhes Dent 2015; 17: 491-497.
17) Leloup G, D’Hoore W, Bouter D, Degrange M, Vreven J. Meta-analytical review of factors involved in dentin adherence. J Dent Res 2001; 80: 1605-1614.
18) Heintze SD, Zimmerli B. Relevance of in vitro tests of adhesive and composite dental materials. A review in 3 parts. Part 3: In vitro tests of adhesive systems. Schweiz Monatschr Zahmmed 2011; 121: 1024-1040.