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ORIGINAL ARTICLE

Using bonding agent prior to pits and fissure sealant application enhances the microtensile bond strength and the interface morphology

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Abstract *Background:* A pits and fissures sealant is an effective method for preventing dental caries. Using a bonding agent before applying the sealant may increase its retention. This study aimed to compare the microtensile strength (μTBS) of a fissure sealant with and without a bonding agent and to characterize the enamel-sealant interface using confocal laser scanning microscopy (CLSM). The null hypothesis was that the use of a bonding agent before fissure sealant application would not change the microtensile strength or the enamel-sealant interface.

Materials and methods: Twenty caries-free premolars were used. Each tooth was divided into four parts. The first two parts were assigned to the bonded group, where a bonding system was used before sealant application. The remaining two parts were treated only with a fissure sealant (i.e., the nonbonded group). In each group, the μTBS was examined after 24 h ($n = 20$) and after a 3-month aging period ($n = 20$). Five other caries-free extracted premolars were used to assess the enamel-sealant interface using CLSM. Two-way analysis of variance (ANOVA) and Pearson chi-square statistical analysis tests were used to analyze the μTBS and the enamel-sealant interface, respectively.

Results: The mean μTBS for the bonded group was significantly higher ($p = 0.001$) in the immediate group (36.87 ± 14.95 MPa) and the aged group (31.08 ± 15.88 MPa) than in the respective

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nonbonded groups (19.77 ± 9.67 MPa and 19.52 ± 14.14 MPa). The µTBS was not significantly different in either group after aging ($p = 0.46$ [bonded group] and $p = 0.98$ [nonbonded group]). In addition, using a dental adhesive, before applying a fissure sealant resulted in a significantly higher (53%) resin penetration into the enamel with the continuous integrity of the resin.

Conclusion: The use of a bonding agent before the application of fissure sealant resulted in superior microtensile bond strength immediately and after aging. In addition, the enamel-sealant interface characteristics were improved.

1. Introduction

Dental caries is a lifestyle-related multifactorial disease process involving a plaque biofilm, which can be controlled by a combination of approaches addressing its etiological factors (Pitts and Wefel, 2009, Chapple et al., 2017). These approaches include diet counseling, physical and chemical oral biofilm control, the use of pits and fissures sealant, and professional topical fluoride application (AAPD, 2008, 2013). A pits and fissures sealant is a material that is introduced to susceptible pits and fissures to establish a tight seal, which prevents the leakage of nutrients to the biofilm in the deeper parts of the fissures (Welbury et al., 2004). The material used is mostly light-activated urethane dimethacrylate (UDMA) or bisphenol A-glycidyl methacrylate (Bis-GMA) resin that bonds to suitable enamel by using the acid-etch technique (Beauchamp et al., 2008). A pits and fissures sealant is most likely to be effective in preventing carious lesions on the occlusal surfaces of primary and permanent teeth (Wright et al., 2016).

A fissure sealant is hydrophobic and technique-sensitive, which requires a high level of saliva control. In some situations, such as newly erupted teeth, maintaining salivary control is very challenging. Feigal et al. (1993) introduced the use of a bonding agent before applying a sealant to increase the retention of the material in teeth contaminated with saliva. However, such a technique can increase the number of clinical steps required to apply the materials and might result in an increase in the chairside time and alter the cost-effectiveness of the sealant material (Tandon et al., 2015).

The success rate of pits and fissures sealants varies, depending on the application technique. Excellent long-term retention of a fissure sealant has been reported (Simonsen, 1991). In that report, 27.6% of pits and fissures sealants were fully retained, and only 10.9% of the sealants were missing after 15 years.

The manufacturers' instructions do not recommend using a fissure sealant with a bonding agent, although studies have supported the use of a bonding agent before applying the sealant (Symons et al., 1996). It was found that such a technique enhances resin penetration into the fissures and increases the retention and bond strength (Torres et al., 2005). This study aimed to compare the microtensile strength of a fissure sealant on natural teeth with and without a bonding agent and to characterize the enamel-sealant interface using confocal laser scanning microscopy (CLSM). The null hypothesis was that the use of a bonding agent before the fissure sealant application would not alter the microtensile strength or the enamel-sealant interface.

2. Material and methods

2.1. Sample preparation

Twenty caries-free premolars were obtained from healthy adult patients aged 18–45 years with the patients' informed consent under a protocol reviewed and approved by the institutional review board at the King Saud University (Riyadh, Saudi Arabia; registration number, E-17-2369). These patients were referred to the Oral and Maxillofacial Clinic of the Dental University Hospital at the King Saud University to extract one or more teeth as part of their regular dental management. All teeth were stored in distilled water at 37 °C and used within a week of extraction. The coronal part of each tooth was obtained by sectioning the tooth through the cementoenamel junction using a slow-speed water-cooled diamond blade (MetLab Technologies, Limited, London, UK). The coronal part was then sectioned into four equal parts (Fig. 1A). Each part was mounted into a custom-made device to stabilize the tooth and expose the buccal or lingual flat surface of the enamel. Then, each part was hand-polished using water-cooled 800-grit sandpaper to ensure the complete flatness of the surface. All exposed surfaces were acid-etched with 35% phosphoric acid for 15 s and then rinsed with water. The samples were gently air-dried to remove excess water without overdrying the enamel.

The first two parts from each tooth were assigned to the bonded group and were treated as follows: (1) bonding using a two-step etch-and-rinse bonding system (Prime & Bond® NT™; Dentsply International, Inc., Charlotte, NC, USA) and (2) applying the fissure sealant (Pits and Fissures Sealant LC; Medental International, Inc., Vista, CA, USA) in a cylinder (2-mm diameter and 3-mm height) by using a custom-made mold (Fig. 1B). The remaining two parts (i.e., the nonbonded group) obtained from each tooth were treated only with the fissure sealant cylinder (Pits and Fissures Sealant LC; Medental International, Inc.). A summary of the materials used in this study, their manufacturer, composition, and mode of application are shown in Table 1.

In both groups, the fissure sealant was applied in two 2-mm increments to ensure complete polymerization (Fig. 1C). The tip of each cylinder was shaped into a funnel so that it could be grasped during testing (Fig. 1D). The bonding system and the fissure sealant increments were activated using the Elipar™ S10 LED curing light (3 M ESPE, St. Paul, MN, USA) (1200 mW/cm^2 intensity) with a wavelength of 430 nm and 480 nm for 20 s and 40 s, respectively. The surface area of the bonding and/or sealant attached to the tooth surface was

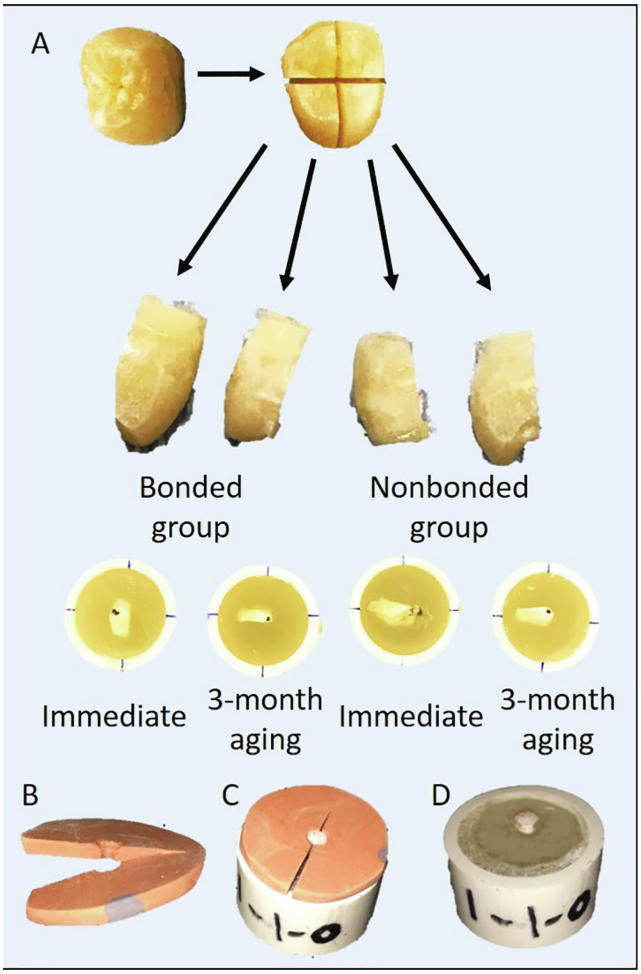

Fig. 1 (A) Schematic representation of how each tooth was sectioned into four parts and the distribution of each part to the study groups; (B) the custom-made mold that was used to create the sealants specimen, a cylinder with 2-mm diameter and 3-mm height; (C) the sealant was applied in two 2-mm increments until the mold was completely filled; (D) the final shape of the specimen is attached to the tooth surface. The top of the specimen is funnel-shaped to allow the specimen to be passively secured with the attachment before testing.

3.14 mm^2. In each group, one sample was tested after 24 h (n = 20). The second sample was tested after a 3-month aging period in distilled water at 37 °C (n = 20).

2.2. Microtensile bond strength

For microtensile bond strength testing, each specimen was interlocked passively to a stainless steel testing apparatus. A tensile load was applied by a universal testing machine (load cell of 5 kN capacity) (model number 5965, Instron, Norwood, MA, USA) at a speed of 1 mm/min. The microtensile bond strength (μTBS) was recorded in MPa. It was obtained by dividing the force (F) required to break the adhesive bond in Newton by the bonding surface area (A) (μTBS = F/A). Each specimen was then evaluated using a Stereo 80 Widefield Microscope (SWIFT Instruments, Inc., Boston, MA, USA) with a magnification power of 10×, with no further specimen preparation, to evaluate the mode of failure.

The mode of failure was determined by the residual sealant on the enamel surface to define the bond failure site. The modes were classified as "pure adhesive mode," "cohesive mode," or "mixed failure mode." Adhesive failure was indicated when breaks existed at the interface between the enamel and the bonding agent or sealant. Cohesive failure was indicated when the failure was predominantly within the sealant. Mixed failure was indicated when part of the sealant remained attached to the enamel. The data for each failure mode was presented as a percentage of the total failures.

2.3. Enamel-sealant interface characterization

Another five caries-free extracted premolars were used for this part of the study. Each sample was stored in distilled water at 37 °C for 24 h. Each tooth was sectioned sagittally into two halves using a slow-speed water-cooled diamond blade (MetLab Technologies, Limited). Each half was then assigned to different groups.

For the bonded group, the adhesive bonding agent was mixed with rhodamine B dye before its application. A 2-mm layer of fissure sealant (Pits and Fissures Sealant LC, Medental International, Inc.) was applied on top after it was mixed with fluorescein dye. For the nonbonded group, only rhodamine B dye was used and was added to the fissure sealant (Pits and Fissures Sealant LC, Medental International, Inc.) before its application.

An additional group, for which the enamel-adhesive interface was assessed alone without the use of a sealant, served as the control group. For this group, three extra teeth were used. The adhesive bonding agent was mixed with rhodamine B dye before its application.

In all groups, the bonding system and the fissure sealant were activated, as described previously. Each sample was sectioned perpendicular to the adhesive-enamel or sealant-enamel interface. The slabs were hand-polished using 400-grit, 600-grit, and 800-grit sandpaper, and underwent ultrasonication for 3 min between each polish.

The slabs were examined using a CLSM (Nikon C2+ System; Nikon Instruments, Inc., Melville, NY, USA) with a 20x/1.4 air objective lens to assess the enamel-sealant interface. For the bonded group, a double labeling technique was used. The slabs were excited with a 561-nm laser to detect rhodamine B dye fluorescence, and the fluorescence signal was detected using 600–630 nm emission filters. Fluorescein was excited at 488 nm, and the emission was detected using a 500–520-nm filter.

The integrity of rhodamine B at the enamel-sealant interface was examined at three preselected areas. Two areas were selected at the periphery of the interface, and one area was in the middle of the enamel-sealant interface. A modified four-scale scoring system, reported by Celiberti and Lussi (2005), was used to analyze rhodamine B integrity as follows: Score 1, resin penetration into the enamel with continuous integrity of the resin; Score 2, resin penetration into the enamel but with phase separation of the resin; Score 3, no resin penetration into the enamel but continuous integrity of the resin; Score 4, no resin penetration into the enamel and phase separation of the resin.

Table 1 The materials used in this study, their manufacturer, composition, and mode of application.

Material	Brand name; manufacturer	Composition	Mode of application
Acid etch	Ultra-Etch$^{\text{TM}}$ etchant; Ultradent Products, Inc., South Jordan, UT, USA	35% Phosphoric acid Highly dispersed Silicon Dioxide ColorantWater	a. Rinse and dry the prepared tooth area. b. Apply the etchant to the enamel for 15 s. c. Rinse the tooth thoroughly and dry it for 10 s without overdrying the enamel.
Bonding	Prime & Bond® NT$^{\text{TM}}$; Dentsply International, Inc., Charlotte, NC, USA	Di- and Trimethacrylate resins PENTA (dipentaerythritol penta acrylate monophosphate) Nanofillers-Amorphous Silicon Dioxide Photoinitiators Stabilizers Cetylamine hydrofluoride Acetone	a. Following acid etching, apply the Prime & Bond® NT$^{\text{TM}}$ adhesive immediately and vigorously to wet the exposed tooth surface only within the custom-made mold using a disposable microbrush. b. Remove the excess solvent by gently drying with clean, dry air from a dental syringe at a distance of 5 mm from the tip for at least 5 s. c. Cure the adhesive for 20 s using a curing light.
Pits and Fissures Sealant	Pits and Fissures Sealant LC; Medental International, Inc., Vista, CA, USA	Bis-GMA (Bisphenol A free) 50–60% Urethane Dimethacrylate (UDMA) 20–30% Triethyleneglycol Dimethacrylate (TEGDMA) 5–10%	a. Apply a 2 mm layer of the sealant within the custom-made mold to adequately cover the flattened tooth surface. b. Remove any air bubbles or voids. c. Cure the sealant for 20 s using a curing light. d. Apply the second layer to fill the entire mold, including the funnel top. e. Cure the second layer of the sealant for 20 s.

2.4. Statistical analysis

Two-way analysis of variance (ANOVA) was used to analyze the microtensile bond strength of the bonded and nonbonded groups. For the failure mode and the enamel-sealant interface characterization, the Pearson chi-square statistical analysis test was used. The significance level (α value) for both tests was 0.05.

3. Results

3.1. Microtensile bond strength

The two-way ANOVA test for microtensile strength testing revealed a significant difference between the bonded and nonbonded groups. However, no significant difference was found in terms of aging. It was found that the mean value of the bonded sealant group was significantly higher ($p = 0.001$) in the immediate group and aged group than in the nonbonded sealant group (Fig. 2). The microtensile strength after aging was not significantly different between the bonded group and the nonbonded group ($p = 0.46$ and $p = 0.98$, respectively). The Pearson chi-square test showed a statistically significant difference in the failure modes for the bonded and nonbonded sealant groups for the immediate period and after the 3-month aging period ($p = 0.04$) (Fig. 3). The incidence of adhesive failure mode (40%) was lower in the bonded immediate group than in the aged samples (Fig. 3). In the nonbonded group, most failures were adhesive-only failures. In both groups, cohesive failure increased significantly after aging (Fig. 3).

3.2. Enamel-sealant interface characterization

Using dental adhesives before the application of fissure sealants resulted in a higher (53%) resin penetration into the enamel with continuous integrity of the resin, compared with the nonbonded group (7%) (Fig. 4). Approximately 50% of the nonbonded group showed no resin penetration into the enamel with phase separation within the resin (Fig. 4). The Pearson chi-square test indicated that the difference between the two groups was statistically significant ($p = 0.045$). A third group was added to the samples for which the enamel-adhesive interface was examined without the application of the fissure sealant material. All samples exhibited resin penetration into the enamel with the continuous integrity of the resin (Score 1). Representative CLSM scans for each score are shown in Fig. 5.

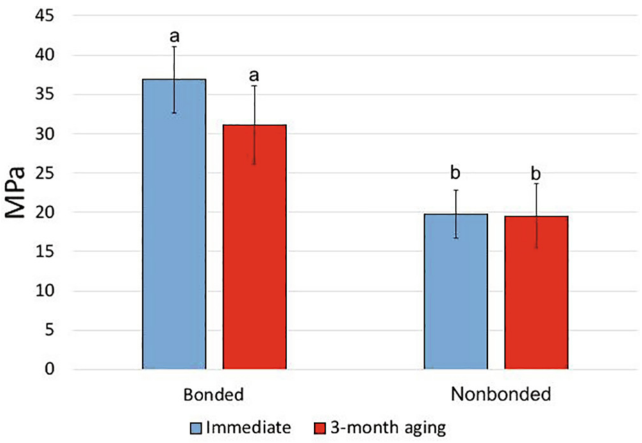

Fig. 2 Mean microtensile bond strength (MPa) of the bonded and nonbonded groups. The bond strength was examined immediately and after a 3-month aging period. Similar letters ("a" and "b") indicate no statistically significant difference ($p > 0.05$). The bars represent the standard error of the mean.

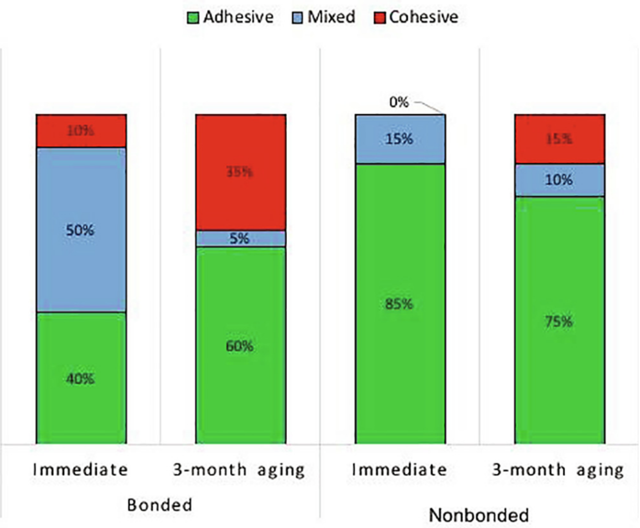

Fig. 3 Failure modes for the bonded and nonbonded groups were examined immediately and after a 3-month aging period. The data are presented as a percentage of each failure mode to the total failures. Adhesive failure is indicated by breaks at the interface between the enamel and the bonding agent or sealant. Cohesive failure is indicated by failure predominantly within the sealant. Mixed failure is indicated when part of the sealant remains attached to enamel.

4. Discussion

This study aimed to determine whether using a bonding agent before the sealant application would affect the sealant's retention, based on microtensile bond strength and interface morphology. We found that applying a bonding agent before applying a fissure sealant resulted in superior microtensile bond strength and improved the enamel-sealant interface.

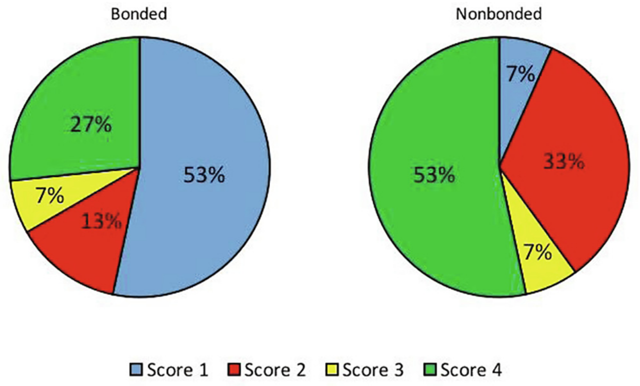

Fig. 4 Percentage of the enamel-sealant interface characterization scores obtained from confocal laser scanning microscopy (CLSM) scans for the bonded and the nonbonded groups. Score 1 indicates resin penetration into the enamel with the continuous integrity of the resin. Score 2 indicates resin penetration into the enamel but phase separation of the resin. Score 3 indicates no resin penetration into the enamel but continuous integrity of the resin. Score 4 indicates no resin penetration into the enamel and phase separation of the resin.

Pits and fissures sealants are accepted as an effective caries prevention method (Kervanto-Seppala et al., 2008). The use of a bonding agent before applying the sealant material is debatable. A recent study (Sen Tunc et al., 2012) reported the effect of using a bonding agent before the sealant application on the microtensile bond strength. Since then, many advances in resin materials have been introduced to enhance bonding and sealant materials. Different bonding systems with different methods of application have been tested previously (Asselin et al., 2009, Meyer-Lueckel et al., 2006).

The initial results of a clinical study by Boksman et al. (1993) showed that, when evaluated six months after placement, a sealant with a bonding agent had a higher retention rate than a sealant without a bonding agent. However, the retention rate did not change after two years when a bonding agent was used. A more recent study by Tandon et al. (2015) demonstrated that the retention of a sealant material did not change when a two-step etch-and-rinse adhesive system was used. The investigators of the aforementioned study concluded that the one-step self-etch system enhanced the retention of sealant material and recommended that it should be used. In addition, a long-term study by Pinar et al. (2005) revealed that the retention rate of a fissure sealant did not change after a 24-month period when a bonding agent was used. Mascarenhas et al. (2008) revealed no change in caries development in teeth sealed with and without bonding agents. The latter study indicated that a sealant should be applied properly with rubber dam isolation, and rubber cup and pumice cleaning to achieve a high retention rate. In general, achieving such an ideal situation is not possible in all clinical cases.

The use of bonding agents between the tooth and fissure sealant can be beneficial to reduce microleakage on saliva-contaminated enamel (Askarizadeh et al., 2008). Furthermore, combining a low-viscosity sealant with a dentin bonding agent has superior microleakage prevention than a high-viscosity sealant with and without the use of a bonding agent (Mehrabkhani et al., 2015). However, other studies found that the use of a bonding agent is not necessary for the prevention of microleakage as well as the enhancement of penetration depth of the sealant material (Marks et al., 2009, Celiberti and Lussi, 2005).

Microtensile bond strength testing has been used widely for the *in vitro* evaluation of the adhesive strength of different dentin bonding systems bonded to the tooth substrate (Armstrong et al., 2010, De Munck et al., 2012, Sen Tunc et al., 2012). In general, immediate and aged microtensile bond strengths are essential to predict the clinical outcome of any adhesive (Van Meerbeek et al., 2010).

Exposure to water is a factor known that degrades tooth-resin bonds (Gwinnett and Yu, 1995). Therefore, storing the specimen in water for a long time serves as a decent method to detect how aging affects the durability and quality of the tooth-restoration interface. Many studies have evaluated the change in bond strength during extended water storage (Burrow et al., 1996, Kato and Nakabayashi, 1998, De Munck et al., 2003). In the current study, the cohesive failure mode increased significantly after aging. This could be explained by the presence of microcracks within the sealant material (Scherrer et al., 2010). In addition, the sealant area that is exposed to water is relatively larger than the bonding material, resulting in more dissolution. However, this does

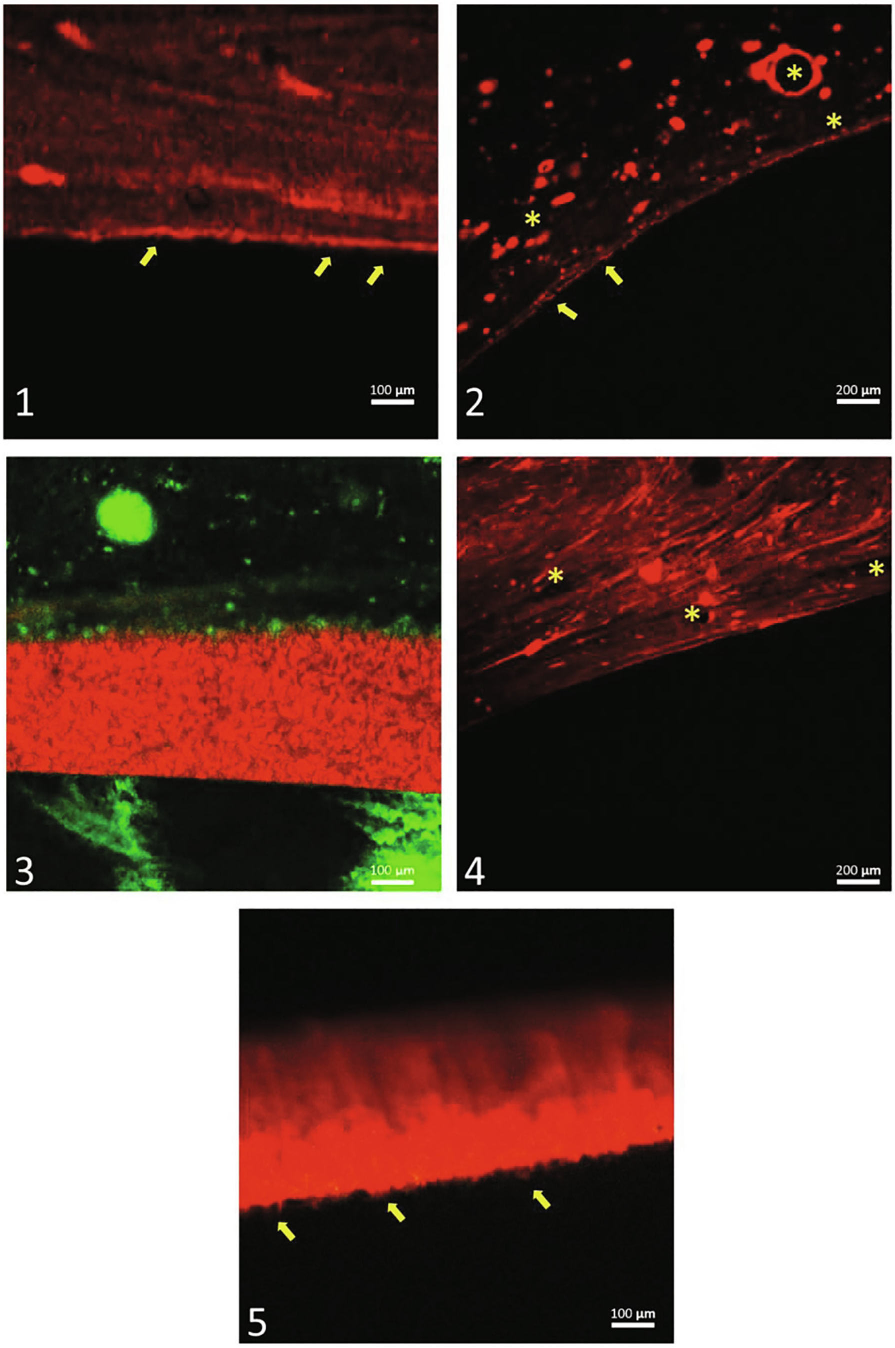

Fig. 5 Representative confocal laser scanning microscopy (CLSM) scans (20x/1.4 air objective lens) for each score used to evaluate the enamel-sealant interface characterization. Image (1) shows the interface with Score 1; image (2), Score 2; image (3), Score 3; image (4), Score 4; and image (5) shows the enamel-adhesive interface without the application of the fissure sealant material. The arrows indicate the resin tags in the enamel surface. The asterisk (*) indicates areas of phase separation of the resin.

not affect the study results, as both bonded and non-bonded groups demonstrate more cohesive failure after aging.

Many studies have indicated that using a bonding agent before applying a sealant will reduce the sealant's ability to penetrate the tooth's grooves and fissures. This phenomenon occurs because of the occlusion of the fissures with a relatively weaker unfilled resin leaving insufficient space for the sealant within the groves. However, by using a low-viscosity acetone-based bonding system with continuous air drying, a thin layer of bonding that is full of the monomer will remain and can adhere chemically to the sealant layer (Naaman et al., 2017).

To reduce the variability among occlusal fissures and to standardize samples, smooth flat surfaces of teeth have been used. In the current study, the microtensile strength results ranged from 20 MPa for the nonbonded group to 30 MPa for the bonded group. This was relatively higher than the microtensile strength results reported previously when a two-step etch-and-rinse bonding system was used (Sen Tunc et al., 2012, Bagheri et al., 2017). This could be attributed to the differences in the chemical contents of the bonding systems used in these studies.

In actual clinical situations, the tensile strength may be higher because of the mechanical interlock within the occlusal surface grooves and fissures.

The significant difference in the microtensile bond strength between the bonded and nonbonded groups in this study was in agreement with the findings of previous studies (Feigal et al., 2000, Feigal et al., 1993, Asselin et al., 2009, Hitt and Feigal, 1992). The use of different types of bonding agents resulting in different microtensile bond strengths has been reported (Sen Tunc et al., 2012).

The microtensile bond strength of 36 MPa for the immediate bonded group was the highest among the groups. In addition, most failures among this group occurred with part of the sealant still attached to the enamel. This finding could be partly explained by the effect of hydrophilic monomers (e.g., 2-hydroxyethyl methacrylate [HEMA]) within the bonding agent, which allows the better spread of the resin material and decreases polymerization shrinkage (Asmussen and Munksgaard, 1985). The late effects of the water absorption of such monomers result in the expansion of the polymer matrix and causes hygroscopic expansion. This increases the volume of the bonding agent and probably decreases the micro gaps created by polymerization shrinkage (Wei et al., 2011). Therefore, the study findings highlight the importance of using an adhesive bonding agent before applying the sealant. In addition, using the bonding agent before applying the sealant resulted in higher resin penetration into the enamel with continuous integrity. Thus, even if part of the clinically detectible fissure sealant was lost, the base of the sealant remains, which has been reported previously (Yun et al., 2013). Therefore, the formation of caries can be prevented.

By contrast, applying a fissure sealant without using an adhesive bonding agent showed lower immediate microtensile strength, with 85% of the failures occurring at the interface between the enamel and sealant. In addition, the interface analysis of this group showed that most samples had no resin penetration into the enamel with phase separation within the resin. This finding can be explained by an increase in internal stresses within the sealant because of polymerization shrinkage. This stress can clinically result in the early loss of the sealant material because of different tensile forces and subsequent formation of caries.

After aging in both groups, no statistical significance was observed within the same group. However, the microtensile bond strength in the bonded groups tended to decrease, which can be explained by a shift in the failure mode; as the rate of adhesive failure increased, the rate of mixed failure decreased. These changes could be related to water sorption and solubility because these factors would affect dimensional instability and the mechanical and bond strength of the resin matrix (McCabe and Rusby, 2004, Bastioli et al., 1990). However, no changes occurred in the microtensile bond strength for the nonbonded aged group because of the constancy of the failure mode, which was primarily within the adhesive interface.

This study has several limitations. The fissure sealant was applied on a smooth surface, which does not resemble the clinical situation. In addition, one type of bonding system was used in this study. Further studies are needed to determine the effect of mechanical interlocking of the occlusal fissures on sealant retention. In addition, different types of bonding systems should be tested for better retention. Furthermore, clinical studies are needed to promote the use of bonding agents before the application of a fissures sealant.

5. Conclusions

The use of a bonding agent before the application of fissure sealant resulted in superior microtensile bond strength immediately and after aging. In addition, the enamel-sealant interface characteristics were improved.

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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Data availability

The data used to support the findings of this study are available from the corresponding author upon request.

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