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ORIGINAL SCIENTIFIC ARTICLE

Using self-etch adhesive agents with pit and fissure sealants. In vitro analysis of shear bond strength, adhesive remnant index and enamel etching patterns

I. Mézquita-Rodrigo[1] · **R. J. Scougall-Vilchis**[1] · **M. A. Moyaho-Bernal**[2] · **L. E. Rodríguez-Vilchis**[1] · **E. Rubio-Rosas**[3] · **R. Contreras-Bulnes**[1]

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Abstract

Purpose The aim of this study was to evaluate in vitro, the shear bond strength (SBS) and adhesive remnant index (ARI) of pit and fissure sealants (PFS) after enamel conditioning with different new-generation self-etching (SE) agents; additionally, enamel etching patterns were assessed.

Methods Healthy unerupted third molars surgically removed for therapeutic reasons ($n = 25$p/g), were randomly assigned to six groups. Conventional etching (CE) or SE was applied prior to pit and fissure sealants bonding. Enamel conditioned surfaces were evaluated by SEM at × 500, × 1000, and × 2000 magnification to determine etching patterns. Subsequently, 25 PFS blocks (3 × 2 × 1.5 mm) p/g were bonded to enamel surface.

Samples were stored in water at 37 °C for 24 h, previous to SBS and ARI test. One-way ANOVA and Tamhane statistic tests were used for SBS; while Mann–Whitney U and Kruskal–Wallis were employed for ARI ($p \leq 0.05$).

Results For SBS test, CE_PFS_3M and SE1_PFS_Shofu groups showed the lowest values (8.74 ± 4.02 and 8.75 ± 3.90, respectively). The highest scores were observed in SE_PFS_Kuraray group (13.46 ± 5.83). Significant differences in SBS and ARI assessments were found. All experimental groups showed type 1 etching pattern.

Conclusion The etching pattern was less pronounced in self-etching groups, which showed an equal or superior in vitro performance compared to conventional etching agents. The clinical use of self-etching agents could be recommended before pit and fissure sealants application in new dental protocols. The best in vitro performance was observed when both applied materials, self-etching agent and pit and fissure sealant have 10-methacryloyloxydecyl dihydrogen phosphate in their chemical composition.

Keywords Self-etching agents · Pit and fissure sealants · Shear bond strength · Adhesive remnant index · Enamel etching pattern

Introduction

The World Health Organization states that caries is the third most prevalent health problem worldwide. Dental caries continues as a significant public health issue across the world. The WHO emphasises that this disease affects about 60–90% of schoolchildren, and one of its policies is to diminish the incidence and prevalence of caries (FDI 2015; Petersen and Ogawa 2016). To decline it, several preventive approaches have been developed, including community water fluoridation, topical fluoride therapy, plaque removal, and diet counselling. However, these measures had a more significant effect on smooth surfaces (Ninawe et al. 2012), and most of the carious lesions occur on the occlusal

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✉ R. Contreras-Bulnes
rcontrerasb@uaemex.mx

[1] Facultad de Odontología, Centro de Investigación y Estudios Avanzados en Odontología (CIEAO), Universidad Autónoma del Estado de México, Jesús Carranza Esq. Paseo Tollocan, Col. Universidad Estado de México, 50130 Toluca, CP, México

[2] Facultad de Estomatología, Benemérita Universidad Autónoma de Puebla, Av. Manuel Espinosa Yglesias 31 Pte. 1304, Col. Los Volcanes, 72570 Puebla, CP, México

[3] Dirección de Innovación y Transferencia de Conocimiento, Benemérita Universidad Autónoma de Puebla, Prolongación de la 24 Sur y Av. San Claudio, Ciudad Universitaria, Col. San Manuel, 72570 Puebla, CP, México

surfaces, which are eight times more susceptible to decay (Bohannan 1983) because of the retentive anatomy of pits and fissures, which represents a challenging condition for cleaning (Ninawe et al. 2012).

Considering this fact, sealants are an important preventive measure for fissure caries prevention (Bohannan 1983); although their documented efficacy and clinical practice guidelines are available, pit and fissure sealants (PFS) are still underused. Furthermore, its retention relies upon the ability of the resin material to thoroughly fill fissures and morphological defects, to remain completely intact and bonded to enamel surface as long as possible (Tay and Pashley 2005; Ahovuo-Saloranta et al. 2008; Erbas et al. 2017; Paglia et al. 2018). Additionally, several factors such as operator's experience, isolation, surface treatment, application of an intermediate bonding layer, among others, could play a role in PFS retention (Erbas et al. 2017).

For this purpose, enamel is commonly etched using 32–37% phosphoric acid (Tay and Pashley 2005). Nevertheless, this conventional etching (CE) method removes several microns from the enamel surface (Vilchis et al. 2007) which could become a disadvantage if sealants lose their retention or marginal integrity from the enamel surface. Some studies claim that the use of adhesive systems as an intermediate layer could improve sealant retention (Bagherian et al. 2016; Botton et al. 2016; Khare et al. 2017; Coelho et al. 2019). Moreover, there is limited scientific evidence comparing effects produced by CE against self-etching (SE) agents before PFS application, especially for 7th generation and universal agents employed in this study.

Therefore, the aim of this study was to evaluate in vitro, the shear bond strength (SBS) and adhesive remnant index (ARI) of pit and fissure sealants (PFS) after enamel conditioning with different new-generation self-etch (SE) agents; additionally, enamel etching patterns were assessed.

Materials and methods

Tooth selection and sample preparation

This in vitro study was reviewed and approved by the Research Ethics Committee of Dental Research, School of Dentistry, at the xxxxxxxxxxx.

A total of 79 third molar teeth surgically extracted for therapeutic indications were obtained under patients' informed consent, with intact enamel surface, no evidence of fluorosis, damage, or fractures were included. The sample preparation was performed by the same operator to enhance the uniformity of the samples. After extraction, teeth were placed in 0.2 (wt./vol) thymol solution; teeth were cleaned with deionised water; traces of soft tissue were removed with a scalpel and stored at room temperature until the experiment was performed.

Each tooth crown was separated from the root using a diamond disc (BesQual, New York, USA) mounted on a low-speed motor (Micromotor M2 Master, M25800011, Drillco Devices Ltd., Miami, FL, USA), irrigating with deionised water to prevent dehydration. The tooth was fixed to a glass slide with thermo-plasticized epoxy resin (Allied High-Tech Products, Rancho Dominguez, USA). Subsequently, a diamond disc (South Bay Technology, Inc., San Clemente, USA) mounted on a trimmer (South Bay Technology, Inc., USA) was used to cut each tooth and obtain two mesiodistal halves. Finally, the samples were thoroughly rinsed with deionised water and randomly assigned according to enamel conditioning protocols in six groups. A Diagram of the experimental design is shown in Fig. 1.

The teeth were mounted in acrylic resin; the sample was abraded on the centre, using a diamond disc to flatten the enamel surface and standardise the area of sealant placement.

Enamel surface treatments

Dental materials employed by each study group are described in Table 1. In all groups, the manufacturer's instructions were followed for product application.

Conventional etching groups (CE)

CE_PFS_3M and CE_PFS_Kuraray. The etchant was applied on the enamel for 15 s, rinsed exhaustively during 15 s with sprayed deionised water and high-pressure air; subsequently, dried with compressed air for 5 s.

Self-etching groups (SE)

SE1_PFS_Shofu. Primer was applied to the enamel surface using a brush, which stood for 5 s. Blown air gently for about 3 s, and then dried with a more substantial draft until a thin, even bond coat was obtained.

SEU_PFS_3M. The adhesive was applied to the prepared tooth and rubbed for 20 s, gently air dry for approximately 5 s to evaporate the solvent and light-cured for 10 s.

SE_PFS_ Kuraray. The adhesive was applied, avoiding rubbing in and left for 10 s, dried with mild air for approximately 5 s, then light-cured for 10 s.

SE2_PFS_Shofu. The adhesive was applied and left for 10 s, gently air dry for approximately 3 s, then blown with more force and light-cured for 10 s.

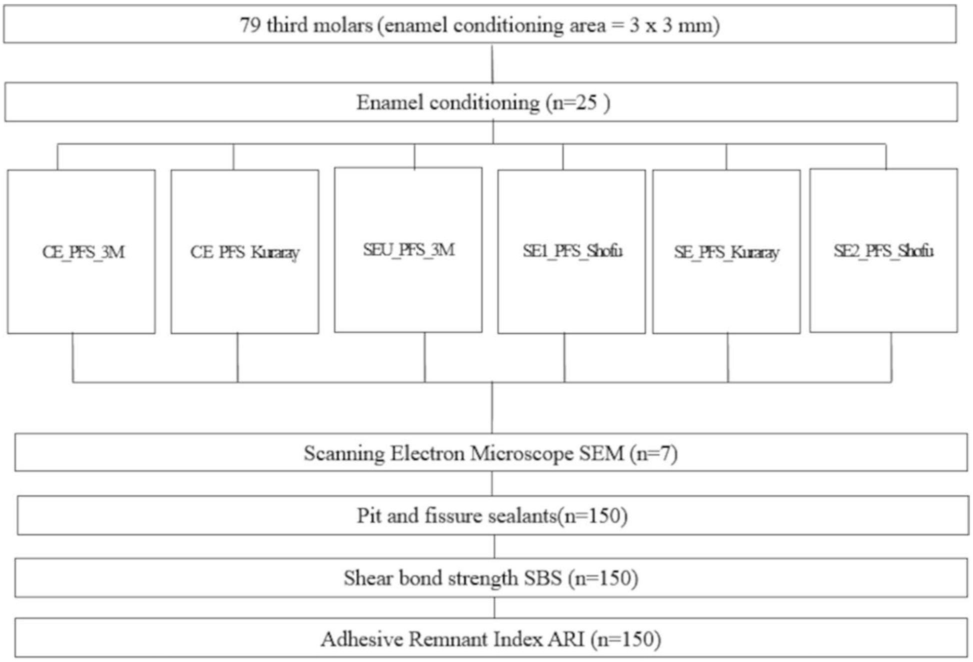

Fig. 1 Diagram of experimental design

Table 1 Study groups

Group	Etching agents	%	pH	Sealant	Manufacturer
CE_PFS_3M	Scotchbond Universal Etchant	32%	0.5	Clinpro	3 M ESPE, St Paul, MN, USA
CE_PFS_Kuraray	K Etchant Gel	40%	1	Teethmate F1	Kuraray, Osaka, Japan
SE1_PFS_Shofu	BeautiSealant Primer	–	*Mild	BeautiSealant	Shofu, Kyoto, Japan
SEU_PFS_3M_PFS_3M	Single Bond Universal	–	2.7 ultra-mild	Clinpro	3 M ESPE, St Paul, MN, USA
SE_PFS_ Kuraray	Clearfil S^3 Bond Plus	–	2.3 mild	Teethmate F1	Kuraray, Osaka, Japan
SE2_PFS_Shofu	BeautiBond	–	2.4 mild	BeautiSealant	Shofu, Kyoto, Japan

Scanning electron microscope (SEM)

To assess the enamel etching pattern (Silverstone et al. 1975) produced by SE agents, six etched samples (without bonded sealant block) were evaluated. Immediately after conditioning. The samples were washed with acetone to dissolve the coating agent and then were dried at room temperature. The samples were fixed to aluminium stubs with adhesive carbon tape (SPI Supplies, USA). The analysis was performed using a scanning electron microscope (JEOL, JSM-6510LV, Japan) in the low vacuum at 10 Pa of chamber pressure, with an electron acceleration voltage of 25 kV and detecting backscattered electrons. Enamel surface morphology and etching patterns were observed at × 500, × 1000, and × 2000 magnification.

Sealant block preparation

A total of 150 sealant blocks (25 p/g) 3 × 2 × 1.5 mm was made and filled into a Teflon mould, covered with a microslide glass and polymerised 20 s with Ortholux Luminous Curing Light (3 M Unitek, Monrovia, Calif., U.S.A.)

Bonding procedure

Finally, each block was bonded on the enamel surface with the corresponding sealant; and light-cured with Ortholux Luminous Curing Light (3 M Unitek) for 20 s. The bonded samples were stored in water at 37 °C for 24 h, previous to the SBS test.

Shear bond strength (SBS)

The specimens were mounted in a universal testing machine to perform the SBS test. Moreover, the mechanical test parameters were established at a speed of 0.5 mm/min until fracture occurrence. Then it was registered in megapascals (MPa) for each sample. The test was accomplished using the flattened end of a steel rod attached to the crosshead of a universal testing machine (Autograph AGS-X, Shimadzu, Kyoto, Japan).

Adhesive remnant index (ARI)

After debonding, the ARI was assessed using the stereomicroscope (Nikon, Tokyo, Japan) at × 10 magnification to determine adhesive remnant on each tooth's enamel surface.

To delimit the area to analyse, a 3 × 2 mm window was cut in the centre of a 9 × 9 mm coloured film tape and marked every 1 mm were carried out around it. This tape guide was delimiting the debonded area. The scores of modified ARI have recorded with the following scale: 0 = no sealant left on the tooth, 1 = less than half of the sealant left on the tooth, 2 = more than half of the sealant left on the tooth, and 3 = all the sealant left on the tooth (Årtun and Bergland 1984). Additionally, to correspond each of these scores to one of the three types of failure previously described in literature by Jain and Stewart (2000), Knobloch et al. (2005) and employed by Coelho et al. (2019) in a similar study, the following criteria were used: adhesive failure: < 20% of remaining sealant; mixed failure: 20–80% of the remaining sealant; cohesive failure: > 80% of remaining sealant.

Statistical analysis

Data were analysed using the SPSS 25 statistical package (SPSS IBM, New York, NY, USA). Kolmogorov–Smirnov test was performed to estimate the distribution of data. The ANOVA test was used to compare SBS into groups; when significant differences were found, Tamhane's T2 post-hoc tests were applied because Levene's test of homogeneity of variance showed different variances. Kruskal–Wallis and Mann–Whitney U tests were used to estimate the differences between ARI groups with a level of significance $p \leq 0.05$ in the complete statistical analysis.

Intra-operator reliability was calculated with Cohen's kappa (0.95), for ARI and etching patterns evaluations.

Results

SEM-etching patterns

Figure 2 shows representative SEM micrographs obtained after enamel surfaces conditioning with different agents. Untreated enamel (a–c) presented a smooth pattern with some enamel grooves. A traditional honeycomb etching pattern (type 1) in CE etching groups was observed (d–i). SE conditioning agents (j–u) also exhibited a type 1 etching

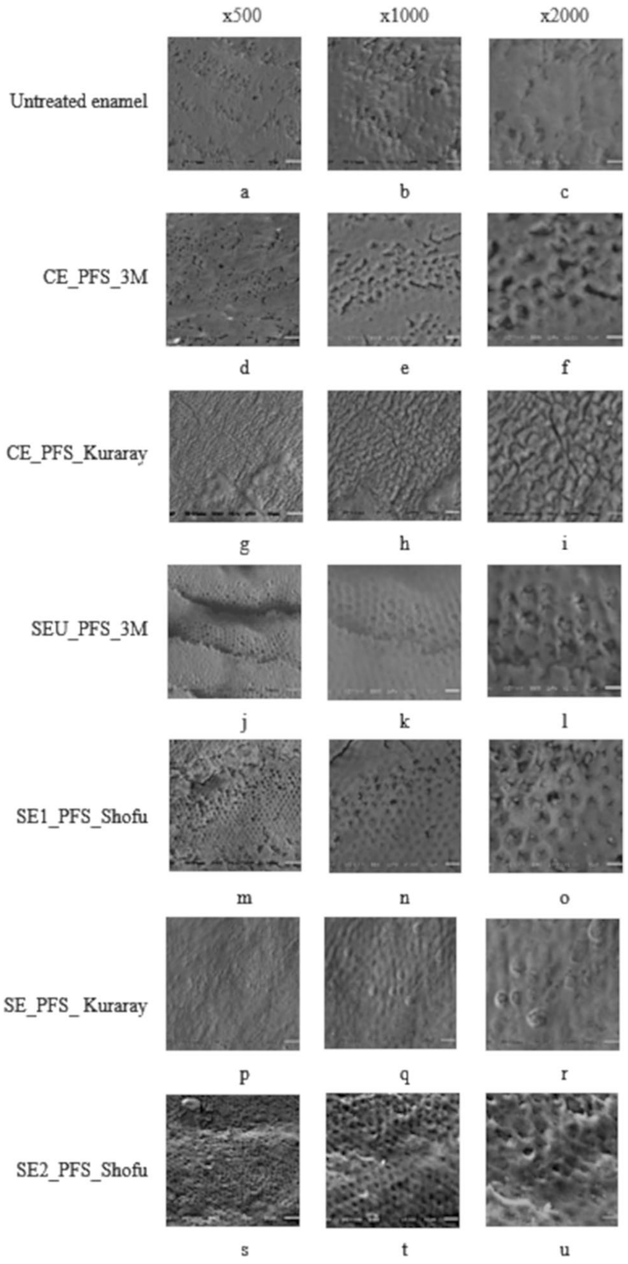

Fig. 2 SEM micrographs after CE and SE conditioning × 500, × 1000, and × 2000 magnification

pattern, with less pronounced exposed prisms, especially for SE_PFS_3M and SE_PFS_ Kuraray groups (p–r).

SBS

Table 2 summarises the descriptive statistics of the SBS values measured, expressed in MPa; CE_PFS_3M and SE1_PFS_Shofu groups showed the lowest values. The highest scores were observed in SE_PFS_Kuraray group, followed by SEU_PFS_3M, SE2_PFS_Shofu, and CE_PFS_Kuraray groups; no significant differences were found among them.

ARI

Analysis of the ARI scores shown in Table 3 illustrates the amount of adhesive remaining after debonding. SE_PFS_ Kuraray was significantly different from all groups ($p \le 0.05$), with percentages above zero for index 2 (8%).

In all groups, most of the failures belonged to the adhesive type, as presented in Table 4. One or two cases of mixed failure were observed in only 2 groups, while any cohesive failure was found.

Discussion

The development and regular use of new adhesive materials have revolutionised many aspects of restorative and preventive dentistry (Sofan et al. 2017). To compare CE against 7th generation SE agents for pit and fissure sealants SBS since the preventive function is achieved mainly by the material adhesion to the enamel surface (Sofan et al. 2017; Carrilho et al. 2019), two concentrations of phosphoric acid (32%

Table 2 Mean bond strength values (MPa) and descriptive statistics

Group	*n*	Mean	SD	Max	Min	*
CE_PFS_3M	25	8.74	(4.02)	17.93	2.63	A
CE_PFS_Kuraray	25	12.08	(6.23)	26.37	3.01	AB
SE1_PFS_Shofu	25	8.75	(3.90)	17.89	2.16	A
SEU_PFS_3M	25	12.69	(6.21)	21.47	2.79	AB
SE_PFS_ Kuraray	25	13.46	(5.83)	29.26	3.39	B
SE2_PFS_Shofu	25	12.25	(7.08)	28.07	2.32	AB

*Tamhane's T2 post- hoc test, $p \le 0.05$. Groups with different letters are significantly different from each other

Table 3 Distribution of adhesive remnant index, frequency and percentage

Group	*n*	0 [%]	1 [%]	2 [%]	3 [%]	*
CE_PFS_3M	25	14 [56]	11 [44]	0	0	A
CE_PFS_Kuraray	25	20 [80]	5 [20]	0	0	AB
SE1_PFS_Shofu	25	21 [84]	4 [16]	0	0	B
SEU_PFS_3M	25	20 [80]	5 [20]	0	0	A
SE_PFS_ Kuraray	25	14 [56]	9 [36]	2 [8]	0	C
SE2_PFS_Shofu	25	23 [92]	2 [8]	0	0	B

*Kruskal–Wallis and Mann–Whitney *U* tests, $p \le 0.05$. Groups with different letters are significantly different from each other

Table 4 Types of failure distribution

Group	*n*	Adhesive [%]		Mixed [%]		Cohesive [%]	
CE_PFS_3M	25	25	[100]	0	[0]	0	[0]
CE_PFS_Kuraray	25	25	[100]	0	[0]	0	[0]
SEU_PFS_3M	25	24	[96]	1	[4]	0	[0]
SE1_PFS_Shofu	25	25	[100]	0	[0]	0	[0]
SE_PFS_ Kuraray	25	23	[92]	2	[8]	0	[0]
SE2_PFS_Shofu	25	25	[100]	0	[0]	0	[0]
Total	150	147	[98]	3	[2]	0	[0]

and 40%) were selected; as well as four self-etching agents (one universal adhesive, and three 7th generation), due to its advantages, as no mixing required ("all-in-one"), consistent bond strengths, humidity control, and clinical time reduction. Although 8th generation agents claim to produce better bond strength, particularly in dentine, stress absorption, and longer shelf life, the nano-fillers particles more prominent than 15–20 nm could cause accumulation in the moisturised surface, forming plugs that produce failures (cracks) resulting in decreased bond strength (Sofan et al. 2017), as an undesirable effect; a reason why they were not included in the present study.

In addition, etching agents from the same brands as PFS were also selected to ensure material compatibility. Roh and Chung (2005) tested SE adhesives in conjunction with resin-based composites from the same and different manufacturers and reported that product combinations from the same fabricator showed higher bond strengths.

In this study, phosphoric acid's effects on SBS were characteristic of the concentration used, showing an SBS directly proportional to the concentration employed (32 and 40%). Furthermore, SE groups showed a slightly higher performance than conventional etching at 32%, and similar to 40% concentration; except for SE1_PFS_Shofu group, which was similar to CE_PFS_3M; and SE_PFS_Kuraray group, which showed a superior efficiency concerning all the groups, probably due to 10-methacryloyloxydecyl dihydrogen phosphate (10-MDP) content of both, self-etching agent and the pit and fissure sealant (Carrilho et al. 2019); considering that, this component is one of the few monomers used in adhesive dentistry, which has been shown to chemically bind to tooth tissues through the ionic bond with calcium found in hydroxyapatite (Yoshida et al. 2012). Although both self-etching agents evaluated in this study had similar pH and the same mild self-etch category, the agent's chemical composition could play an important role in adhesion.

However, previous reports have concluded lower retention for sealants applied with SE systems than sealants applied in the conventional approach, regardless of the use of adhesive systems (Botton et al. 2016). Nevertheless, most of the SE agents studied in the cited systematic review and meta-analysis correspond to the 6th generation, and there are scarce reports in the relevant literature related to the associate use of 7th generation SE and PFS. In this regard, Pitchika et al. (2018) studied SBS for fissure sealing with the same SE adhesive and PFS employed in SE1-PFS-Shofu study group, finding lower values (4.6 MPa) than us; notwithstanding, the PFS blocks had different characteristics (size and form), which could influence load force distribution, and therefore, the SBS.

Moreover, there are no previous reports regarding SBS of PFS associated with the use of universal agents. Ataol et al. (2017) determined and compared resin-based PFS's ability bonded to enamel with an intermediate layer of universal adhesives, finding that there was a significant reduction in microleakage compared to conventional PFS placement. The universal single bond agent evaluated in this study exhibited the highest resistance to microleakage than the BeautiSealant Primer at seven days of water storage and self-etch mode when Clinpro and BeautiSealant PFS were applied, respectively. Both products combinations used in this study revealed higher SBS values for Universal Single Bond and Clinpro (SEU_PFS_3M) than those for BeautiSealant Primer and BeautiSealant (SE1_PFS_Shofu). Additionally, a previous study evaluated SBS for BeautiSealant Primer and BeautiSealant application according to manufacturer instructions reported 4.6 MPa at 1-day water storage (Pitchika et al. 2018), a lower value than found in this research work. It is essential to notice that 5.9–7.8 MPa resistances are enough to resist masticatory forces (Reynolds 1979), and all our study groups obtained higher values to withstand these forces. However, universal adhesives' sealing effectiveness under resin-based PFS appears to be material-dependent (Ataol et al. 2017). Even though the gold standard of adhesives are etch and rinse materials until 2019, the current trend prefers simplified bonding procedures (Takeda et al. 2019). In this order, universal adhesives have different advantages over previous generations of adhesives because they can be used for several restorative procedures, multiple substrates, and adhesion strategies; they also have the ability to bond chemically to hydroxyapatite provided by their acidic monomers (Perdigão and Swift 2015).

Also, 10-MDP integrated into the composition of most universal adhesives, bonds ionically, as previously mentioned. The hydrolytically stable calcium salts on hydroxyapatite results in nano-layers. This chemical bonding to the enamel crystallites of etched enamel might increase the short- and long-term enamel bond strength, according to Zhang et al. (2013). Regardless of ionic adhesion, SEU_PFS_3M group showed lower SBS values than the SE_PFS_Kuraray group, probably due to Single Bond Universal's ultra-mild pH compared to the mild pH of Clearfil S3 Bond Plus, which could produce a stronger enamel etching. However, further study is required to clarify this finding, considering that 10-MDP is also found in PFS chemical composition.

Furthermore, another advantage of using SE or universal agents is a more conservative etch pattern as observed in this study, it has been explained due to a reduced enamel loss (-0.03 to -0.74 µm), contrary to the effect of conventional etching (-1.11 to -4.57 µm) (Hosein et al. 2004). Nevertheless, it is necessary to study enamel loss according to new etching agents and current procedure times, specific to the dental substrates.

Concerning the adhesive remnant index (ARI) system, it was selected to assess the amount of adhesive left on the enamel surface tooth because it is a quick and straightforward method that needs no special equipment (Montasser and Drummond 2009). Since ARI's introduction in Orthodontics (Årtun and Bergland 1984), the index has had multiple uses and evaluations (Montasser and Drummond 2009), as in this study for PFS.

For ARI assessment, the experimental groups presented different adhesive remnant patterns, although almost in all cases, the same brand groups were similar, as results observed in 3 M and Shofu groups. Contrary to Kuraray groups, where the use of conventional etching agent was similar to the other products while using a SE agent, only this group presented higher rates of remaining adhesive (score 2). It could be derived from the adhesive effect produced by combined characteristics as the presence of 10-MDP in both the SE agent and PFS and the etching effect produced by the pH of the products. According to ARI scores, SE_PFS_Kuraray group could avoid alterations in the dental substrate, such as fractures or loss of enamel, in the case of detachment.

Furthermore, the failure results of this study coincide with a mainly adhesive failure reported by Sen Tunc et al. (2012) and Coelho et al. (2019), although their studies were carried out in bovine teeth, unlike ours, in human teeth.

The failures reported by Sen Tunc et al. (2012) were consistent with the type of PFS and/or adhesion system used, all failures were adhesive type in the groups which employed 5th or 6th generation SE agents, previous to PFS placement from a specific brand; although, adhesive systems and PFS trademark were not similar to each other, which could influence their results.

Coelho et al. (2019) also reported a higher type of adhesive failure when evaluating the adhesion of PFS after enamel conditioning with 6th generation self-etching agents; however, the adhesion performance and therefore the type of failures produced were related to enamel contamination (saliva or water) and SE agent composition (ethanol or water-based adhesive), obtaining better results under ideal conditions of uncontaminated, such as those evaluated in this study.

Nevertheless, Bishara et al. (2001) consider as a clinical advantage that bond failure does not occur at the enamel-adhesive interface, to prevent enamel surface damage. In addition, when there is partial loss of the sealant, the remaining material still will exist on the surface of the tooth. Pérez-Hernández et al. (2018) mentioned that sealants have to remain complete in the surface of the tooth, for this reason it is recommended to check patients frequently to verify the sealant's status.

Although all treated groups showed an etching pattern corresponding to type 1, with prism core material preferentially removed, leaving the prism peripheries relatively intact, a more pronounced etching pattern appeared in CE groups, especially for CE_PFS_Kuraray group, probably due to the higher concentration of phosphoric acid (40%). In contrast to CE, SE groups showed a more conservative type 1 etching pattern. SEM observations suggest that the etching ability of the different agents employed could be related to their pH.

Furthermore, the etched enamel with 10-MDP containing groups (SEU_PFS_3M and SE_PFS_Kuraray) shows an enamel surface with a fused appearance, probably due to the stable and intensive interaction with hydroxyapatite that this component produces, as has been reported in the literature (Carrilho et al. 2019). As expected, untreated enamel presented an even surface.

A limitation of this study was the in vitro design, which could not reproduce all possible in vivo oral conditions; such as undesired effects, including contamination by humidity, saliva or blood, among other circumstances as the patient's behaviour and approach. Furthermore, the difficulty of comparing the results obtained with those of previous reports, due to the multiple methodologies employed or the fast progress of the dental materials.

Therefore, considering that SE agents produced a gentler etching pattern on the enamel surface, as well as enhanced SBS values; it would be convenient to study the enamel surface porosity generated by these agents, for a better understanding of their adhesion mechanisms, as well as microleakage at the enamel–PFS interface.

Dentistry is always in constant evolution for the search of new and better prevention and restoration alternatives, as well as for patients and dentist protection. Even considering the limitations of this in vitro research, the results obtained could withstand the use of SE agents, in the face of the COVID-19, the current pandemic due to their advantages, since they reduce the risk of cross infections derived from the elimination of the rinsing step, reduction time of possible exposure to pathogen agent, as well as saving dental chair time, a variable especially desired in the case of paediatric dentistry patients.

Conclusions

- The etching pattern was less pronounced in self-etching groups, which showed an equal or superior in vitro performance according to shear bond strength and adhesive remnant index, compared to conventional etching agents.
- The clinical use of self-etching agents could be recommended before pit and fissure sealants application in new dental protocols.
- The best in vitro performance was observed when both applied materials, self-etching agent and pit and fissure sealant have 10-methacryloyloxydecyl dihydrogen phosphate in their chemical composition.

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Data availability Data available on request from the corresponding author.

Declarations

Conflict of interest The authors have no conflicts of interest to declare.

Ethical approval The study was carried out according to the principles of the Declaration of Helsinki and was approved by the Institutional Review Board as mentioned in materials and methods section.

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