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Research article

Micro-tensile bond strength of two pit and fissure sealants to intact enamel

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ABSTRACT

Background: Sealing dental pits and fissures with resin-based sealants effectively prevents occlusal dental caries. The effectiveness of resin-based pit and fissure sealants relies on maintaining a strong bond between the sealant and the enamel.
Objective: This *in-vitro* study compared the micro-tensile bond strength (μTBS) of a conventional resin-based sealant (Clinpro™) and a hydrophilic resin-based sealant (Embrace™ WetBond™) when applied to intact, aprismatic human enamel.
Methods: Forty extracted permanent premolar and molar teeth were divided into two groups and paired by tightly approximating two buccal surfaces to create an artificial enamel groove (fissure). Fissure sealants (Clinpro™ and Embrace™ WetBond™) were applied to the artificial enamel 'grooves' in each group. The specimens were then cut into beams with a cross-sectional area of 1 mm^2 and tested for the micro-tensile bond strength (μTBS). Fractured surfaces of samples were examined under a conventional microscope to identify the failure modes. Two specimens from each group were prepared and observed under a scanning electron microscope (SEM). Mann-Whitney U and Fischer-Freeman-Holton exact tests were used to test the statistical differences between the fissure sealants.
Results: The μTBS mean ± SD for Clinpro™ was 16.43 ± 7.08, and 10.57 ± 6.64 for Embrace™ WetBond™. There was a statistically significant difference in μTBS between Embrace™ WetBond™ and Clinpro™ ($p < 0.001$). There was no association between fissure sealant and failure modes ($p = 0.922$).
Conclusion: Clinpro™ showed higher μTBS to enamel than Embrace™ WetBond™. Further studies are needed to conclude the clinical effectiveness of these sealants.

1. Introduction

The morphological complexity of teeth, such as occlusal pits and fissures, makes them highly susceptible to caries [1].

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Table 1
List of materials.

Material	Manufacturer	Composition	Enamel conditioning
Clinpro™	3 M ESPE, St. Paul, MN, USA	• Bisphenol A Diglycidyl Ether Dimethacrylate (BISGMA) •2,2′-ethylenedioxydiethyl dimethacrylate •2-Propenoic acid, 2-methyl-, 3- (trimetoxysilyl)propyl ester, hydrolysis products with silica •Tetrabutylammonium tetrafluoroborate •Diphenyl Hexafluorophosphate •Ethyl 4-Dimethylaminobenzoate (EDMAB) •Titanium dioxide •Triphenylantimony •Hydroquinone	Absolutely dry
Embrace™ WetBond™	Pulpdent Corporation Watertown, MA, USA	•Acrylate ester monomers in two-part, glass-filled, dual-cure paste •Uncured acrylate ester monomers •Silica, amorphous •Sodium fluoride	Relatively dry
Spident Fine Etch	Spident Co., Ltd, South Korea	•phosphoric acid (37%), •bio-compatible polymeric materials	

Epidemiological data show that pit-and-fissure surfaces carry a significant amount of the total caries burden in schoolchildren [2]. Preventive measures such as fluoride toothpaste and topical fluoride application have limited effectiveness against occlusal caries; therefore, physical preventive approaches such as pit and fissure sealants are currently considered the most effective [3].

Sealing pits and fissures of primary and permanent teeth can reduce the incidence of dental caries [4]. Applying pit and fissure sealants improves mechanical cleansing of teeth and reduces the surface area of the tooth exposed directly to demineralisation [5].

Resin-based pit and fissure sealants are the most used material for sealing pits and fissures [6–13]. Evidence suggests that placing resin sealants keeps the sealed occlusal surfaces caries-free and prevents the development of cavitated carious lesions in high-caries-risk individuals [14].

The effectiveness of sealants in preventing caries relies on their clinical retention over time [6,15–17], which in turn requires stable and strong adhesion (bonding) between a sealant and the enamel [8]. A dry tooth surface is a necessary condition for the application of a resin-based sealant to ensure effective adhesion of the sealant to the enamel [8,13,18–20].

Traditional pit and fissure sealants, such as Clinpro (Clinpro™), require a completely dehydrated enamel surface for effective bonding, whereas newly developed moisture-tolerant sealants, such as Embrace (Embrace™ WetBond™), require a lightly dried but not completely desiccated enamel surface [15,21–23].

The newly developed fissure sealants thus offer an advantage over the traditional sealants since achieving and maintaining a completely desiccated enamel surface is more time-consuming and challenging than achieving a relatively dry surface [19,21–23]. This difference becomes particularly significant when treating paediatric patients, children of special needs and other potentially uncooperative patients [24].

In-vivo studies have evaluated the retention rates of different fissure sealants. These studies found no statistically significant difference between the retention rates of the conventional sealant Clinpro and the moisture-tolerant sealant Embrace [15,25,26]. However, limited studies have been conducted to test the effectiveness of different sealant types based on their retention and comparative bonding strengths [27–29].

The micro-tensile bond strength (μTBS) test is the best *in-vitro* measure of dental resin-based adhesive restorative retention [27,30]. The μTBS test is a reliable *in-vitro* testing method for evaluating experimental variables in adhesive bonding materials to the tooth structure. The μTBS test method is relatively easy to perform, could be repeatable within and between laboratories, and is ultimately helpful in predicting clinical outcomes [28]. Ideally, adhesion to tooth structure should provide retentive strength and marginal seal, be relatively simple to achieve and have clinical durability. The μTBS test, especially after subjecting the specimens to a durability challenge, is currently recommended as the best surrogate measure of dental composite restoration retention [29].

Although the bond strengths of some fissure sealants have been compared [31], to date, no study has compared the micro-tensile bond strengths to the enamel of conventional (Clinpro) and moisture-tolerant (Embrace) sealants.

Therefore, this in-vitro study aimed to measure and compare bond strengths of conventional (Clinpro) and hydrophilic (Embrace) pit and fissure sealants. Both Clinpro and Embrace are currently available on the market. Clinpro, like all conventional sealants, requires a perfectly dry enamel surface. On the other hand, Embrace, a hydrophilic sealant, requires a moist enamel surface for bonding.

The study hypothesis proposed no difference between the micro-tensile bonding strength (μTBS) of Clinpro and Embrace bonded to intact enamel when tested soon after the bonding.

2. Materials and methods

Forty sound permanent premolar and molar teeth extracted for orthodontic reasons from patients aged 20 to 40 were obtained from dental clinics across Auckland. The teeth were cleaned and separately stored in distilled water at 4 °C with the details of the extraction date to be used within one month following extraction. Teeth were then removed and polished with pumice to remove any surface

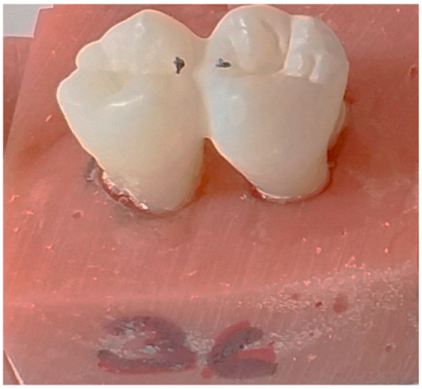


Fig. 1. Sealed teeth embedded in a resin block.

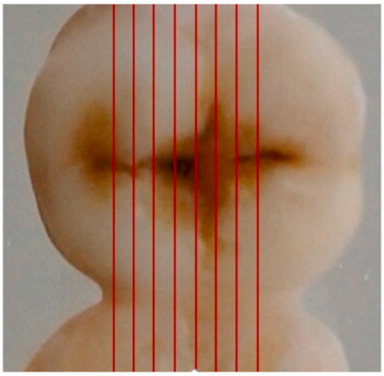


Fig. 2. Tooth sectioning (parallel lines).

debris or contaminants. Then, they were stored in 0.1% thymol solution at room temperature before the experiment.

The teeth were not subjected to thermocycling or ageing because this study intended to compare the bond strengths immediately after the bonding, as the technique of using the two sealant materials was different.

This study adopted and modified the simplified enamel fissure model for a more realistic representation of a fissure [12] because occlusal morphology is an essential factor in the depth of penetration of the sealant into the enamel [32].

2.1. Teeth preparation

The roots of all teeth were trimmed below the cementoenamel junction (CEJ), leaving about one-third of the roots. The crowns were randomly assigned to one of the two groups ('Clinpro' or 'Embrace'), differing in the procedure employed for enamel preparation and the type of sealant used (Table 1). Each tooth was secured with sticky wax on a stable surface. The selected surfaces of two teeth were tightly approximated to create an artificial occlusal 'groove' using a spacer to a mean distance of 0.6 ± 0.1 mm (Fig. 1). Next, all teeth were conditioned with 37% phosphoric acid and rinsed with distilled water. The teeth were then air-dried according to the manufacturer's instructions for the type of sealant used. Fissure sealants were applied to the groove and light-cured (with SDI Radii Plus LED curing light) for 20 s. After applying the sealant, each tooth was removed from sticky wax and embedded in acrylic resin (Unifast Trad II, GC, Tokyo, Japan), making a block of roughly 1.5cm x1.5 cm in dimension. The roots of the teeth were embedded in the resin block, with the crowns bonded to sealant visible from the CEJ (Fig. 1).

2.2. Specimen preparation

The teeth were stored in distilled water at room temperature for less than 24 h before sectioning. Then, they were sectioned with the slow-speed diamond saw featuring a diamond-coated blade (Isomet, Buehler, Lake Bluff, IL, USA). Each tooth was sectioned perpendicular to the bonded surface under copious water cooling to obtain 1.00 mm thick slices (Fig. 2). Each slice was further sectioned into beams with a sealant interfacing surface area of 1.00 mm2 (Fig. 3).

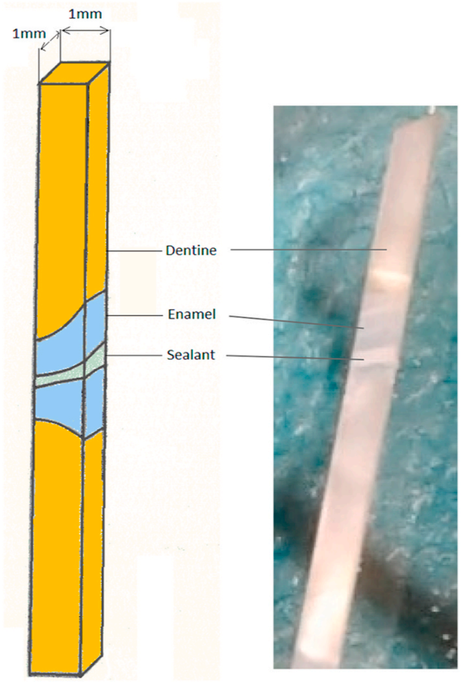


Fig. 3. A specimen with a sealant surface area of 1.00 mm^2.

2.3. *Testing the micro tensile bond strength*

A single operator performed individual testing of each specimen. Before the testing, all specimens were examined with an optical microscope (Leica ICC50 HD) (magnification ×30) for defects at the bonding interface. Specimens showing defects such as apparent interfacial spaces, formations of air bubbles, or any other imperfections were removed and replaced with sound specimens. The specimens were fixed to opposing arms of the testing device (TA.XT.plus Texture Analyser by Stable Micro Systems) using Cyanoacrylate adhesive (Selleys Quickfix Liquid Supa Glue). Each specimen was fractured under tension at a crosshead speed of 1 mm/min. The maximum load (peak force) at the moment of fracture was measured in Newtons and recorded.

2.4. *Failure modes*

After μTBS testing, the fractured samples were examined under a conventional microscope with a camera attachment (Leica ICC50 HD) at an original magnification of ×40 to explore and categorise the failure modes. The failure modes were sorted into one of the following four types: (1) adhesive failure between the fissure sealant and the enamel, (2) cohesive failure in the enamel, (3) cohesive failure in the fissure sealant, (4) mixed failure.

2.5. *SEM observation*

Two specimens from each group were selected for SEM observation. Four bonded enamel/sealant specimens were cross-sectioned at the interfaces after storing for 24 h at 37 °C to observe the interfaces between enamel and the adhesive resin. The specimens were polished with polishing sheets in a descending sequence up to 3-μm roughness. They were then etched with 0.1 N HCl for 30 s, washed with distilled water, and dried in an incubator for 24 h. Finally, the surfaces were sputter-coated with platinum using a Hitachi E−1045 sputter coater and observed under a scanning electron microscope (Hitachi SU-70).

2.6. *Statistical analysis*

Ultimate stresses in MPa were calculated as a ratio of the peak force (N) to the surface area of 1.00 mm^2. The mean ± SD for μTBS (in MPa) was calculated. A Mann-Whitney *U* test was run to determine if differences existed between the μTBS measured for each sealant. Frequencies and percentages were determined for modes of failure. A Fisher-Freeman-Halton exact test was conducted between sealant type and failure modes. A p-value <0.001 was considered statistically significant. Statistical analyses were performed with the SPSS software (IBM Corp. Released 2020. IBM SPSS Statistics for Windows, Version 27.0. Armonk, NY: IBM Corp).

Table 2
μTBS to enamel (mean ± SD) in MPa.

Specimens for μTBS test	μTBS (mean ± SD)	p-value
Clinpro™ specimens N = 53	16.43 ± 7.08	0.001
Embrace™ WetBond™ specimens N = 59	10.57 ± 6.64	

Note. **p*-value calculated using Mann-Whitney *U* test for continuous variables.

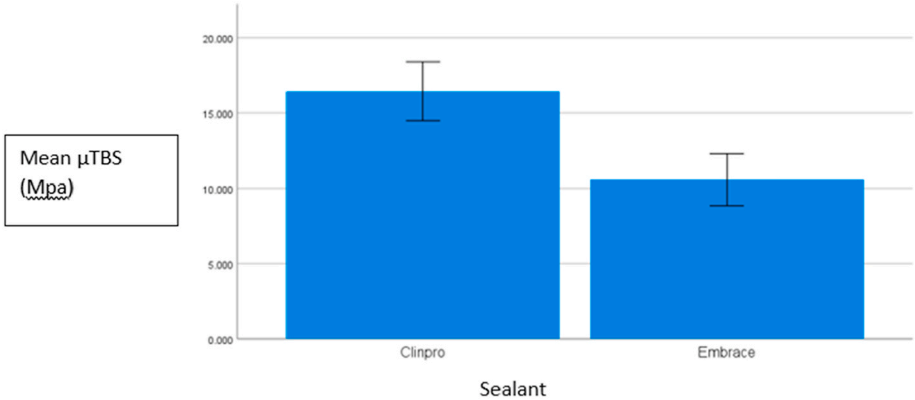


Fig. 4. µTBS (MPa) for Clinpro™ and Embrace™ WetBond™.

Table 3
Modes of failure following µTBS.

Specimens identifying modes of failure	Adhesive n (%)	Cohesive in the enamel n (%)	Cohesive in the sealant n (%)	Mixed n (%)	*p*-value
Clinpro™ specimens N = 59	10 (16.9)	2 (3.4)	32 (54.2)	15 (25.4)	0.922
Embrace™ WetBond™ specimens N = 59	12 (20.3)	2 (3.4)	28 (47.5)	17 (28.8)	

Note. n = failure modes frequencies; % = n/NX100.
**p*-value calculated using Fisher-Freeman-Halton exact test for categorical variables.

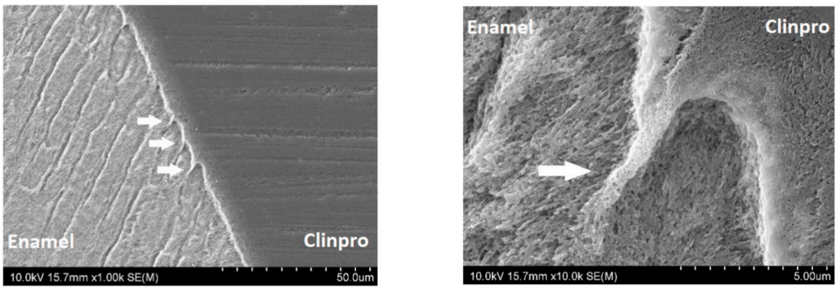


Fig. 5. 5.1 and 5.2: SEM images of the adhesive interface of Clinpro™ bonded to enamel showing resin tag-like penetrations of about 10 µm into the enamel (arrows).

3. Results

3.1. Micro tensile bond strengths and failure modes

The µTBS (in MPa) mean ± SD for Clinpro and Embrace are shown in Table 2 and Fig. 4. The mean µTBS and ±SD of Clinpro was 16.43 ± 7.08 and 10.57 ± 6.64 for Embrace. There was a statistically significant difference in µTBS between Embrace and Clinpro at p < 0.001.

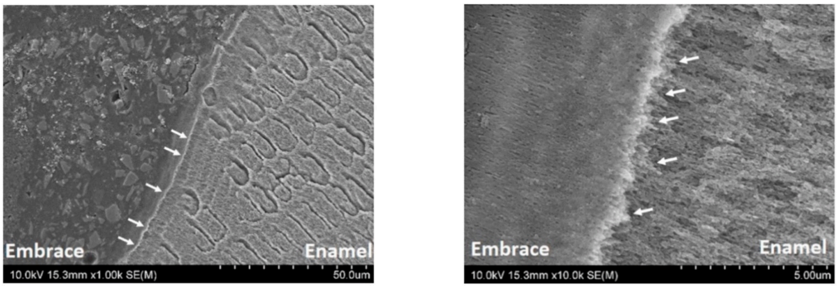


Fig. 6. 6.1 and 6.2: SEM images of the adhesive interface of Embrace™ WetBond™ bonded to enamel showing irregular resin tag-like penetrations of about 5 μm into the enamel (arrows).

Of 224 samples, 118 were analysed to detect the failure modes. The frequencies and percentages of failure modes following the μTBS test are summarised in Table 3. The most common failure mode was cohesive in the sealant (54.2% for Clinpro and 47.5% for Embrace). There was no association between fissure sealant and failure modes as assessed by the Fisher-Freeman-Halton exact test, $p = 0.922$.

3.2. SEM observations

SEM photomicrographs of the adhesive interface between enamel and the sealants are shown in Figs. 5.1 and 5.2 (Clinpro), and in Figs. 6.1 and 6.2 (Embrace). There is no discontinuity in the enamel-resin interface in either sealant system, although the Embrace–resin bonding interface exhibited a interrupted interface in some areas. Images of 10k magnification of the interface showed about 10 μm resin tags in the hybrid layer with Clinpro and about 5 μm with Embrace. Pictures of the bonding interface of Embrace showed an irregular pattern of resin tags at the interface, unlike demarcated tags in Clinpro.

4. Discussion

Most of the studies on μTBS examined the effect of ageing on the retention or bond strength, but this study compared the bond strength (μTBS) of newly placed sealants that were not subjected to ageing. One study concluded that the results of the micro tensile or shear bond strength of different fissure sealants bonded to enamel were not influenced by the thermal cycling of the specimens [33].

The present *in-vitro* study showed a significant difference in the μTBS between the two commercially available resin-based sealants – Clinpro, a conventional (Bis-GMA-based) sealer and Embrace, a hydrophilic (acrylate monomer-based) sealant ($p < 0.001$). Weaker values of μTBS were observed for Embrace, using a wet bonding technique, compared to Clinpro, using a conventional bonding technique, when measured soon after bonding to enamel.

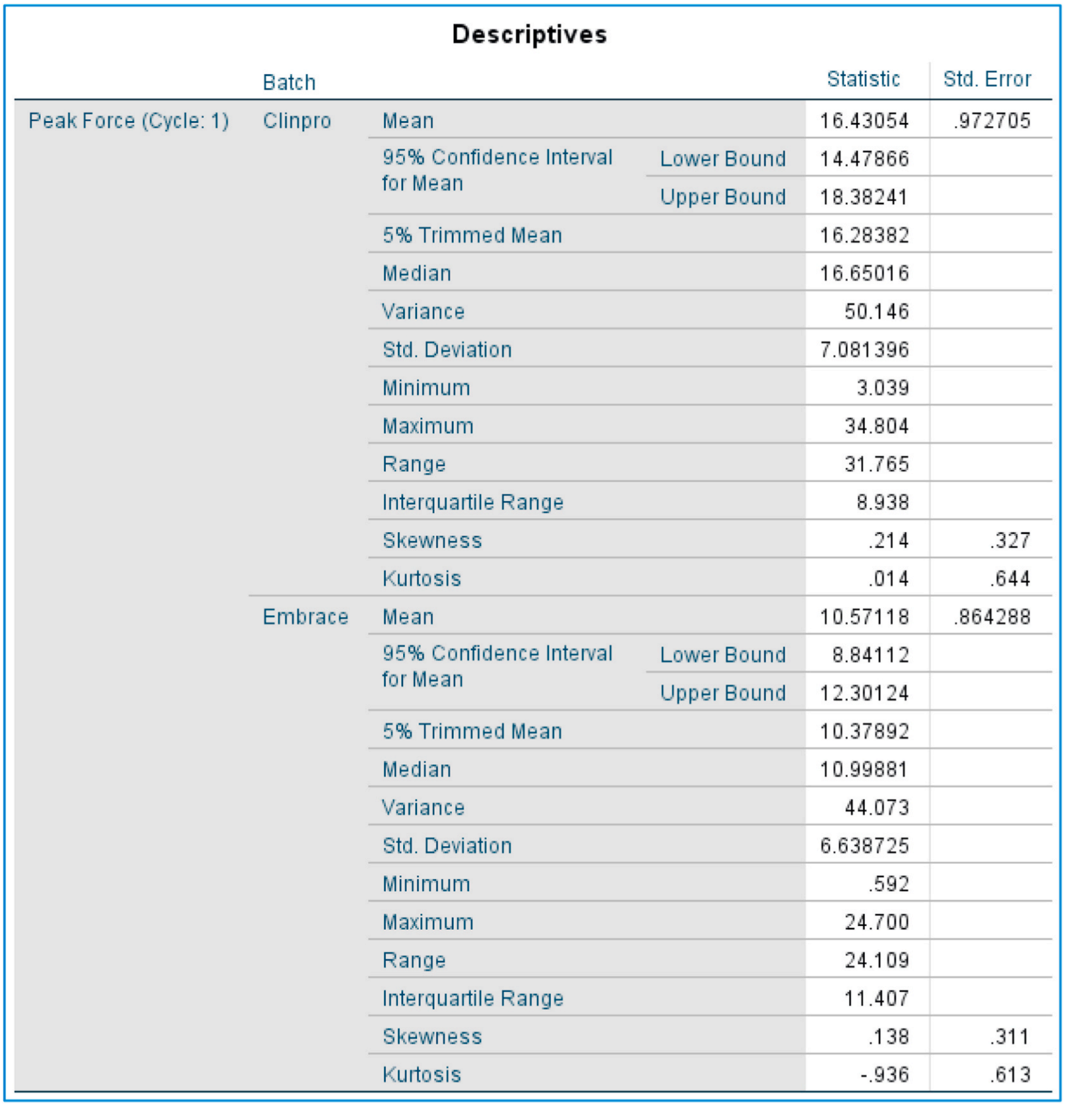
Similar studies have been conducted on different sealant products, comparing their μTBS [31]. An *in-vivo* study [15] found that the conventional resin-based and hydrophilic resin-based sealants showed similar retention rates after a follow-up period of 6 months, and after 12 months, the rate of completely retained sealants was 72.7% for the Embrace; in contrast to the studies by Bhatia [25] and Subramanian [26], which showed the 12-months complete retention rates for Embrace sealants as 23% and 10%, respectively. However, none of the three studies showed a statistically significant difference between the retention rates of the conventional sealant Clinpro and the moisture-tolerant sealant Embrace.

The success of sealants is technique-sensitive, as most products require maintaining a dry field. A study showed that Embrace had demonstrated reliability over time after being wet bonded [15]. Another study that compared the marginal adaptation under SEM of the sealants Clinpro and Embrace found that the marginal adaptation of Embrace was statistically superior to Clinpro [24]. This finding is in sharp contrast to the present study, where it was found that under high magnification (10K), SEM samples bonded with Embrace sealant had multiple areas where the sealant was stripped away from the enamel surface. The tag-like penetrations into the enamel were also irregular and inconsistent in samples bonded with Embrace under the SEM.

Al-Quahtani et al., 2022 compared the μTBS of bioactive resin-based sealants (Bio-RBS) and resin-based sealants (RBS) applied to primary and permanent teeth with and without bonding agents. They concluded that when applied to permanent teeth, Bio-RBS exhibited superior μTBS compared to RBS [34].

The null hypothesis was rejected in the present study. Rejecting the null hypothesis may mean that the wet bonding technique using Embrace sealant does not provide the anticipated bonding strength soon after the bonding procedure is completed.

In conclusion, this study provided baseline information for the μTBS of Clinpro, a traditional sealant requiring a completely dry tooth surface and Embrace, a hydrophilic sealant requiring a moist tooth surface for bonding. Moreover, it studied the bonding surfaces under SEM for two sealants. It is appropriate to say that there is a need for further studies to compare the Embrace, a hydrophilic sealant, to other available pit and fissure sealant materials, not only in the μTBS but also in the micro-shear bond strength, micro-leakage, and longevity. The bonding of the sealant application techniques on different uncontaminated, saliva-contaminated, and water-contaminated enamel conditions is another factor that could be investigated. Moreover, there is a need for clinical studies to evaluate the long-term success and retention of Clinpro and Embrace. Although inferior to Clinpro, the bonding strength achieved with



Descriptives

	Batch			Statistic	Std. Error
Peak Force (Cycle: 1)	Clinpro	Mean		16.43054	.972705
		95% Confidence Interval for Mean	Lower Bound	14.47866	
			Upper Bound	18.38241	
		5% Trimmed Mean		16.28382	
		Median		16.65016	
		Variance		50.146	
		Std. Deviation		7.081396	
		Minimum		3.039	
		Maximum		34.804	
		Range		31.765	
		Interquartile Range		8.938	
		Skewness		.214	.327
		Kurtosis		.014	.644
	Embrace	Mean		10.57118	.864288
		95% Confidence Interval for Mean	Lower Bound	8.84112	
			Upper Bound	12.30124	
		5% Trimmed Mean		10.37892	
		Median		10.99881	
		Variance		44.073	
		Std. Deviation		6.638725	
		Minimum		.592	
		Maximum		24.700	
		Range		24.109	
		Interquartile Range		11.407	
		Skewness		.138	.311
		Kurtosis		-.936	.613

Fig. 7. Descriptive statistics.

Embrace may still be adequate for the long-term retention of the sealant when used in patients. Therefore, further clinical trials would be recommended to evaluate the long-term retention of the two types of sealants.

4.1. Limitations of the study

As this was an *in-vitro* laboratory study, only the μTBS of the bonding of two sealants was studied soon after the sealant placement. An in-vivo (clinical) study will need to investigate the clinical application and retention of the sealants over time.

4.2. Clinical relevance

Fissure sealants are routinely applied as a preventive measure globally. Clinpro and Embrace are two commercially available sealants used in oral health clinics. Research on the bond strengths would provide more information about the two sealants and their retention ability over time.

Ethics statement

- This study was reviewed and approved by **the Health and Disability Ethics Committee** and **the Auckland University of Technology Ethics Committee**, with approval numbers **HDEC-17/STH/226** and **AUTEC-18/250.**

Clinical significance

Fissure sealants are routinely applied as a preventive measure globally. Clinpro™ and Embrace™ WetBond™ are commercially available sealants used in oral health clinics. Research on the bond strengths would provide more information about the two sealants and their retention ability over time.

Data availability statement

Data associated with the study has not been deposited into a publicly available repository. Data has been included in the appendix section of the article (Fig. 7).

CRediT authorship contribution statement

Erekle Sesiashvilli: Writing – review & editing, Writing – original draft, Visualization, Resources, Investigation, Data curation. **Priyadarshanee M. Ratnaweera:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Project administration, Methodology, Investigation, Formal analysis, Conceptualization. **Claudia Zagreanu:** Writing – review & editing, Validation, Resources, Formal analysis.

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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Adrian Owens: Senior Technician. Department of Applied Science, AUT.

tored in distilled water at room temperature for less Meie Zou: Senior Technician - Teaching. Department of Applied Science, AUT.

Tim Layt: Laboratory and Health & Safety Manager, School of Science, AUT.

Yuan Tao: Research Officer - SEM. Department of Design & Creative Technologies, AUT.

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