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Sealant and composite bond strength to enamel with antibacterial/self-etching adhesives

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Summary. *Introduction.* The enamel in pits and fissures undergoing preventive or ultraconservative resin restorations may be affected by secondary caries. An antibacterial adhesive bond may be a useful choice to prevent and reduce demineralization.

Objectives. The purpose of this study was to determine the shear bond strength of an experimental antibacterial bond, a self-etch bond, and an ormocer-based adhesive bond using an ormocer-based fissure sealant and a composite resin on intact enamel.

Methods. Twenty-four extracted human molars were sectioned bucco-lingually to obtain two flat and sound enamel surfaces. Forty-eight specimens were randomly assigned into four groups. The groups were prepared to receive the following treatments: (1) Admira Bond + Admira Seal ($n = 10$); (2) ABF Bond + Admira Seal ($n = 11$); (3) Clearfil SE Bond + Clearfil AP-X ($n = 12$); and (4) ABF Bond + Clearfil AP-X ($n = 11$). A cylindrical mould was placed over the bonded surface, and both materials were applied according to the instructions. The teeth were thermocycled 500 cycles between 5 and 55° with a dwell time of 30 s. For shear bond strength testing, the load was applied vertically to the base of the mould. The cross-head speed was 5 mm min^{-1}.

Results. The maximum load that a specimen can withstand until failure was determined and shear bond strength was calculated by dividing the load at failure by the surface area of the mould. The results (median, minimum and maximum, respectively) for the four groups were: (1) 7·9, 4·2 and 13·6 MPa; (2) 8·6, 2·6 and 15·9 MPa; (3) 5·9, 5·3 and 8·2 MPa; and (4) 8·8, 3.0 and 19·2 MPa. Kruskal–Wallis and Mann–Whitney *U*-tests were performed for the statistical analysis. Groups 3 and 4 were statistically significantly different ($P = 0{\cdot}005$; $P < 0{\cdot}05$). There was no statistically significant difference between the sealant and composite groups for ABF ($P = 0{\cdot}375$). Fracture sites were viewed by microscope under × 16 magnification to determine if the mode of failure was adhesive or cohesive. The results (number of adhesive failed specimens and number of cohesive failed specimens, respectively) for the four groups were: (1) 7 and 3; (2) 3 and 8; (3) 2 and 9; and (4) 8 and 3.

Conclusion. The ABF bond has sufficiently physical qualities to be used under fissure sealant and composite resin on uncut enamel, its antibacterial efficiency notwithstanding.

Introduction

The development and spread of preventive dentistry over the past 20 years has contributed to a decline in the prevalence of clinically detectable dental caries, especially in developed countries. There has been a

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notable fall in the number of proximal lesions, whilst the occlusal surfaces have continued to be the most susceptible to this disease [1,2]. Consequently, the utilization of a physical and an antibacterial barrier in the form of a pit and fissure (P&F) sealant, which isolates the occlusal surfaces from the oral surrounds in order to impede the onset of caries, has a valid scientific foundation [3,4].

Resin-based P&F sealants are usually placed after cleansing and phosphoric acid etching of the fissure enamel [5,6]. However, the highest bond strengths to human enamel were attained using phosphoric acid etching and adhesives underneath the sealants [7–9].

Contemporary adhesives can be divided into two systems in term of clinical application: etch and rinse (E&R) adhesives and self-etch adhesives [10]. The first system uses phosphoric acid etching and primer/adhesive resin in one bottle, and the latter combines etching and priming in one bottle and an adhesive resin in another, or uses etch-prime-adhesive all in one procedure.

Bonding to enamel with the E&R system is a reliable technique. The self-etching adhesives use hydrophilic, acidic monomers which are able to demineralize and penetrate enamel and dentin simultaneously [11]. This system represents a simplification of the bonding procedure and a potential decrease in technique sensitivity. Therefore, self-etching adhesives may be effective in sealing pits and fissures, and this simplified method dramatically shortens treatment time and its complexity.

Remineralization by release of fluoride from P&F sealants is representative [12,13], but the antibacterial effect is another important property because inactivation of bacteria means a direct strategy to eradicate the cause of dental caries. The antibacterial effects of dentin bonding systems indicate the inhibition of caries formation, especially along the enamel margins [14,15]. Recently, an experimental fluoride-releasing antibacterial adhesive system, ABF, was developed by combining the physical advantages of dental adhesive technology and an antibacterial effect. An experimental self-etching adhesive system, ABF incorporates 12-methacryloyloxydodecyl-pyridinium bromide (MDPB), which is an antibacterial monomer [16,17]. An adhesive bond possessing antibacterial properties may be advantageous underneath sealant materials.

The purpose of this *in vitro* study was to determine the shear bond strength of an experimental antibacterial self-etching primer system, a self-etching primer system and an ormocer-based total-etch adhesive system on intact enamel under an ormocer-based fissure sealant and a resin composite.

Materials and methods

Twenty-four extracted human molars were collected. They were examined with the naked eye, and determined to be free of surface cracks, decalcification or any sign of previous grinding. The teeth were stored in saline solution at room temperature immediately after extraction and were used within 2 weeks. The enamel surfaces were cleaned with a pumice-flour slurry and rubber cup for 10 s and rinsed for 5 s. No other preparation was performed. The teeth were sectioned bucco-lingually to obtain two separate portions. Each portion of the tooth was placed into a plastic ring and embedded in methylmethacrylate so that the flattest proximal enamel surfaces would be parallel to the horizontal plane. Forty-eight specimens were randomly assigned to four groups. Each group contained 12 specimens. The groups were prepared to receive the following treatments (Table 1):

Group 1. Ormocer-based, one-component adhesive system + ormocer-based P&F sealant:
Admira Bond (Voco) + Admira Seal (Voco)

Group 2. Experimental fluoride-releasing, self-etching, antibacterial adhesive system + ormocer-based P&F sealant:
Experimental ABF (Kuraray) + Admira Seal (Voco)

Group 3. Self-etching adhesive system + microhybrid composite resin:
Clearfil SE Bond (Kuraray) + Clearfil AP-X (Kuraray)

Group 4. Experimental fluoride releasing, self-etching, antibacterial adhesive system + microhybrid composite resin:
Experimental ABF (Kuraray) + Clearfil AP-X (Kuraray)

The adhesive systems were applied on intact enamel surfaces (Table 1). A special cylindrical mould with a diameter of 2·7 mm was placed over the bonded surface, and both the fissure sealant and the resin composite were placed following the application directions of the manufacturers (Table 1). The teeth were subjected to 500 cycles of thermocycling between 5 and 55° with a dwell time of 30 s. To test shear bond strength, the specimens were held

Table 1. Materials and application procedures used in this study.

Material	Chemical composition	Component	Procedure
ABF (Kuraray)	MDP, HEMA, MDPB, water MDP, HEMA, NaF, microfiller	ABP KBF	Apply ABP for 20 s, air-dry Apply KBF, light-cure for 10 s
SE Bond (Kuraray)	MDP, HEMA, water BIS-GMA, MDP, HEMA microfiller	Primer Bond	Apply Primer for 20 s, air-dry Apply Bond, light-cure for 10 s
Vococid (Voco)	34·5% PA	Etching gel	Apply for 30 s, rinse for 30 s, thoroughly dry
Admira Bond (Voco)	Ormosers, aromatic and aliphatic dimethacrylates BIS-GMA, HEMA, BHT, acetone, organic acids	Bond	Apply, let act for 30 s, air-dry Light-cure for 20 s
Admira Seal (Voco)	Ormosers, aromatic and aliphatic dimethacrylates, BHT, 54% inorganic filler (0·7 μm)	Sealant	Apply, let penetrate for 20 s, light-cure for 30 s
AP-X (Kuraray)	Ba filler, BIS-GMA, TEGMA	Hybrid composite	Light cure for 40 s

in jaws which had been clamped to the base plate of a universal testing machine (Zwick Test Machine, Zwick GmbH & Co., Ulm, Germany). A shear load was applied vertically by a knife-edged rod from the load cell to the base of the cylindrical mould. The cross-head speed was 5 mm min^{-1}. The maximum load that the specimen could withstand until failure was determined and shear bond strengths were calculated by dividing the load of failure to the surface area of the mould. After testing shear bond strength, the fracture sites were then viewed by microscope under × 16 magnification (Leica MS5, Wetzlar, Germany) to determine if the mode of failure was either adhesive or cohesive (four specimens were lost during the strength testing and one specimen was accidentally lost during the microscopic examination).

Statistical analysis was accomplished by Kruskal–Wallis and Mann–Whitney *U*-tests. The mode of failure data were evaluated by chi-square test.

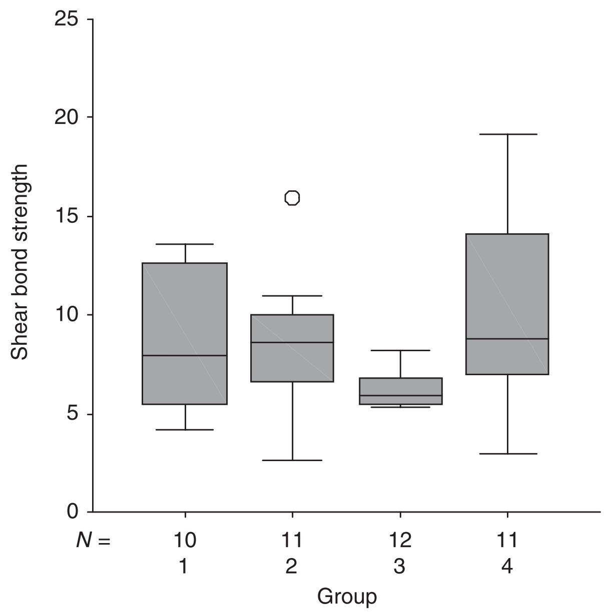

Fig. 1. Shear bond strengths of the adhesive groups.

Results

There was no statistical difference between experimental antibacterial self-etching adhesive (ABF) + sealant group and total-etch adhesive (Admira Bond) + sealant group (Table 2). Groups 2 and 4 reached a median shear bond strength of more than 8 MPa (Fig. 1). There was significant difference between SE Bond and the composite group, and between ABF and the composite group ($P = 0{\cdot}005$; $P < 0{\cdot}05$). An analysis of the failure mode at the fracture sites showed that the self-etching sealant group revealed generally more cohesive (73%) failures in contrast to the total-etch sealant group ($P = 0{\cdot}015$; $P < 0{\cdot}05$) (Table 3).

Table 2. Enamel shear bond strengths of the four adhesive groups investigated in this study.*

Group	Number	Median (MPa)	Minimum (MPa)	Maximum (MPa)
1	10	7·9[a]	4·2	13·6
2	11	8·6[a]	2·6	15·9
3	12	5·9[b]	5·3	8·2
4	11	8·8[a]	3·0	19·2

*Groups identified with the same subscripts are not significantly different ($P > 0{\cdot}05$).

Discussion

The efficacy of correctly applied fissure sealants in the prevention of caries has been demonstrated, and their use has increased over recent years in both

Table 3. Distribution of modes of failure.*

Group	Total number	Adhesive failure		Cohesive failure	
		Number	Percentage	Number	Percentage
1	10	7	70	3	30
2	11	3	27	8	73
3	11	2	18	9	82
4	11	8	73	3	27

*$\chi^2 = 10{\cdot}442$, $P = 0{\cdot}015$.

individuals and community programmes [18]. Sealants can be a very effective way of preventing caries in pits and fissures in primary and permanent teeth [19–21]. The placement of sealants is a painless and noninvasive technique that avoids unnecessary preparation of the tooth structure. However, rinsing the tooth after acid etching can be unpleasant and may become a source of disruptive behaviour, particularly in young children [21].

It has been reported that a hydrophilic bonding agent used without contamination yielded bond strengths which were significantly greater than the bond strength obtained when using sealant alone without contamination [22,23]. It has been proposed that the dentinal adhesive systems may improve the retention rate of sealants in deep fissures, particularly if the fissure is not completely dry prior to resin placement [24].

While most of the data from the above studies seem to favour the use of a layer of bonding agent as part of the sealant procedure, Boksman *et al.* carried out a clinical trial of sealants with and without bonding agent, and found no benefit to the use of the bonding agent with respect to retention rate [5]. Use of a bonding agent would tend to increase the time (and cost) of the sealant application procedure, and thus, should be carefully weighed before adoption [25].

Bonding to enamel is achieved by micromechanical adhesion resulting from the diffusion of resin monomers into the treated enamel and polymerization of resin creating a hybrid-like layer in the enamel [26–28]. The length of resin tags has been shown to contribute little to the bond strength of resin to enamel, and bonding is mainly attributable to the ability of the resin to penetrate between the enamel crystallites and rods [27,28].

Scanning electron micrograph investigations of current self-etching adhesives have shown enamel etching patterns which are morphologically similar to that of phosphoric acid-etched enamel [29]. Nevertheless, the achievement of a sufficient etching pattern on unground enamel still remains a problem for self-etching adhesives [27].

The intact enamel surface is prismless, hypermineralized and contains more inorganic material than the inner enamel layer. However, the effect of a self-etching primer system is less influenced by the orientation of the prismatic structure of enamel than that of a one-bottle adhesive system [30].

Since the shear strength testing method is not applicable to the morphology of occlusal surfaces, the bonding performance of the current adhesive systems underneath the sealants could be evaluated on the intact enamel surfaces. In this study, the shear bond test enabled the evaluation of the bond strength of adhesives on flat surfaces of intact approximal enamel. Shear bond strengths for all the groups in the present study were much lower than those reported by Barkmeier *et al.* and Fuks *et al.* [31,32]. The different bond strengths obtained in those studies may be related to the variations in the recommended application technique.

In most previous studies, investigators have ground enamel surfaces using silicon carbide paper prior to the application of adhesive resins for bond tests [27,33–38]. In Ibarra *et al.*'s study [39], the microtensile bond strength of self-etching adhesives to ground and unground enamel was found to be similar, but it has recently been shown by Perdigao *et al.* [40] that self-etching primers produce high-tensile bond strengths when enamel is roughened, but lower tensile bond strengths when enamel is left unprepared.

The results of an *in vitro* study may not be directly extrapolated to clinical situations since the shearing strength test cannot be performed on irregular occlusal surfaces. Moreover, we do not know what the lowest acceptable adhesion value is, i.e. the lowest value at which sealants can be retained [32].

Feigal emphasized that it is important to remember that all sealants exhibit partial loss in the strict sense of the term because all show reduced volume over time [41]. The extent of sealant changes in volume and area have been documented *in vivo* [42,43]. Changes become clinically significant when sealants have lost enough material to leave a deep fissure uncovered, or when sealants fracture and the sharp marginal defect may lead to caries.

An antibacterial adhesive system underneath the sealant will also exhibit antibacterial action on the cariogenic microflora of the original pits and fissures, and on the caries formation that may occur after microleakage or a partial loss of the sealant.

The ABF experimental self-etching adhesive system was developed by combining the antibacterial agent quaternary ammonium and a methacryloyl group [44]. Imazato *et al*. have been conducting investigations on the utilizations of an MDPB since 1995, and have reported the incorporation of MDPB into the self-etching primer [45,46]. MDPB copolymerizes with other monomers after curing and the antibacterial agent is covalently bonded to the polymer network. The immobilized agent does not leach out from the material, but acts as a contact inhibitor against the bacteria which attach to the surface [47]. The above authors have reported a number of findings concerning *in vitro* antibacterial activity, bonding ability, cytotoxicity and pulpal response of a MDPB-containing self-etching primer/adhesive [45–49]. In addition, they have confirmed that MDPB-containing primer could show antibacterial effects *in vivo* using animal models, and the usefulness of the antibacterial adhesive systems has been validated [50].

Basically, ABP has been developed from SE Bond incorporating 5% MDPB into its self-etching primer (ABF). MDPB has been found to be able to completely kill the bacteria obtained from the clinical carious lesions. Clearfil SE Bond and the experimental ABF adhesive are two-step self-etching primer systems with a low pH (pH = 2).

Another study of dental bonding systems reported that antibacterial effects were produced at low pH [51,52]. However, the bactericidal activities of self-etching primers elicited at a low pH are not reliable since they are ineffective against acid-tolerant bacteria such as lactobacilli [52].

It has been reported that the bond strength of the filled sealant was higher than or equal to that of the unfilled sealant [53]. Stavridakis *et al*. [54] showed that high-viscosity sealant material performed well when used in combination with the self-etching primer adhesive system as an intermediate layer.

In this study, it was found that the median bond value of Clearfil SE Bond with Clearfil AP-X resin composite was significantly lower than that of experimental ABF Bond with Clearfil AP-X resin composite. Clearfil SE Bond led to a statistically lower enamel bond strength value than the other three groups. The higher enamel bond strength of ABF to enamel than SE Bond may be attributed to its different contents. It is interesting to note that, once Admira Seal is used with an antibacterial self-etching adhesive system, i.e. ABF, shear bond strength values are similar to those of an E&R adhesive system.

The cohesive failure in the fissure sealant or resin composite indicates that the adhesion forces between the enamel and the ABF are stronger than the cohesive forces of the composite and fissure sealants themselves.

What this paper adds
- This report describes bond strengths of a sealants and a composite to enamel using 2 different adhesive systems.
- An experimental fluoride releasing anti-bacterial self etching system gave shear bond strengths that appeared to be a little higher- but not statistically significant-to those of a conventional self-etch.

Why this paper is important for paediatric dentists
- This paper highlights the continued development of adhesive systems which may provide additional preventive benefits in clinical practice.

Conclusion

Under the limitations of this *in vitro* study, the total-etch and experimental antibacterial self-etching adhesives used underneath the sealant provided similar bond strengths. The ABF bond is sufficiently physically qualified to be used under fissure sealant and resin composite on intact enamel, its antibacterial efficiency notwithstanding. However, further clinical research is needed to confirm these *in vitro* results.

Résumé. *Introduction.* L'émail des puits et fissures sous des restaurations préventives ou ultraconservatrices en résine peut être le site de caries secondaires. Un agent adhésif anti-bactérien peut être un choix utile afin de prévenir et réduire la déminéralisation. *Objectifs.* Le but est de déterminer la force de résistance à l'arrachement d'un agent adhésif expérimental, auto-mordançant anti-bactérien à base ormocer, utilisant un scellant de sillons à base ormocer et une résine composite sur de l'émail intact.
Méthodes. 24 molaires humaines extraites ont été sectionnées bucco-lingualement pour obtenir 2 surfaces amélaires saines les plus plates possibles. 48 spécimens ont été répartis au hasard en quatre groupes. Les groupes ont été préparés pour recevoir les traitements suivants: Group 1 : Admira Bond + Admira Seal (n = 10); Groupe 2 : ABF Bond + Admira Seal (n = 11); Groupe 3 : Cleafil SE Bond + Clearfil AP-X (n = 12); Group 4 : ABF Bond + Cleafil AP-X (n = 11). Un moule cylindrique a été placé sur la surface préparée avec l'agent et les deux matériaux

ont été appliqués selon les instructions. Les dents ont été thermocyclées 500 cycles entre 5° et 55°, les échantillons étant en place 30 secondes. Pour tester la résistance à la traction, la charge été appliquée verticalement à la base du moule. La vitesse de la tête était de 5 mm/mn.
Résultats. La charge maximum avant arrachement a été déterminée et la force d'arrachement a été calculée en divisant la charge d'arrachement par la zone de surface du moule. Les résultats ont été (médiane, minimum, maximum respectivement) : Groupe 1 (7,9 MPa; 4,2; 13,6), Groupe 2 (8,6 MPa; 2,6; 15,9), Groupe 3 (5,9 MPa; 5,3; 8,2) Groupe 4 (8,8 MPa; 3; 19,2). L'analyse statistique a été réalisée par les tests de Kruskal-Wallis et de Mann-Whitney. Les groupes 3 et 4 étaient statistiquement différents (p = 0,005; p < 0,05). Il n'y avait pas de différence statistique entre les groupe scellant et composite pour ABF (p = 0,375). Les sites de fracture ont été observés au microscope sous une magnification de 16X afin de déterminer si le mode de fracture était adhésif ou cohésif. Les résultats ont été : (nombre de spécimens avec échec adhésif ; nombre de spécimens avec échec cohésif) pour les groupes 1 (7; 3), 2 (3, 8), 3 (2, 9), 4 (8, 3).
Conclusion. La liaison ABF est physiquement suffisamment qualifiée pour pouvoir être utilisée, en plus de son efficacité anti-bactérienne, sous un scellant de sillon et une résine composite sur de l'émail intact

Zusammenfassung. *Einführung.* Schmelz unter präventiven oder ultrakonservativen kompositrestaurationen kann von Sekundärkaries betroffen sein. Ein antibakerielles Adhäsiv könnte nützlich sein, vorzubeugen und Demineralisierung zu reduzieren.
Ziele. Ziel dieser Studie ist es die Haftfestigkeit eines experimentellen antibakteriellen Adhäsivs festzustellen im Vergleich zu einem Ormocer und einem Komposit Adhäsiv.
Methoden. 24 menschliche extrahierte Molaren wurden in bukkolingualer Richtung getrennt, um zwei flache Oberflächen gesunden Schmelzes zu erzielen. 48 Proben wurden zufällig vier Gruppen zugeordnet. Die Gruppen wurden vorbereitet für folgende *Behandlungen.* Gruppe 1 : Admira Bond + Admira Seal (n = 10); Gruppe 2 : ABF Bond + Admira Seal (n = 11); Gruppe 3 : Clearfil SE Bond + Clearfil AP-X (n = 12); Gruppe 4 : ABF Bond + Clearfil AP-X (n = 11). Eine zylindrische Form wurde über die mit Schmelzoberfläche plaziert, beide Materialien wurden nach Herstellervorschrift angewandt. Die Zähne wurden 500 Zyklen zwischen 5° und 55° thermisch wechselbelastet mit einer Äquilibrierungszeit von 30 s. Zur Messung der Scherkraft wurde die Kraft vertikal zur Basis der Form appliziert. Die Scherklingengeschwindigkeit lag bei 5 mm/min.
Ergebnisse. Die maximale Probenbelastung wurde ermittelt und die Scherkraft errechnet aus der Oberfläche der Form dividiert durch die Kraft bei Debonding. Die Ergebnisse waren: (Median; Minimum; Maximum) Gruppe 1 (7.9 MPa; 4.2; 13.6), Gruppe 2 (8.6 MPa; 2.6; 15.9), Gruppe 3 (5.9 MPa; 5.3; 8.2) Gruppe 4 (8.8 MPa; 3; 19.2). Kruskal-Wallis und Mann-Whitney-Tests wurden durchgeführt die Unterschiede zwischen Gruppe 3 und 4 waren statistisch signifikant (p = 0.005) Es lag keine statistisch signifikante Differenz vor zwischen den Gruppen mit Versiegeler und Komposit mit ABF (p = 0.375). Bei lichtmikroskopischer Untersuchung der Bruchflächen unter 16facher Vergrößerung auf die Bruchart wurden adhäsive bzw kohäsive Bruchmodi gefunden für Gruppe 1 (7;3) 2 (3;8), 2 (2;9), 4 (8;3).
Schlussfolgerung. ABF Bond ist hinsichtlich der Haftwerte stark genug, um unter Versiegelungen oder Komposit an intaktem Schmelz eingesetzt zu werden neben der antibakteriellen Wirkung.

Resumen. *Introducción.* El esmalte de las fosas y fisuras bajo restauraciones de resina preventivas o ultraconservadoras pueden estar afectadas por caries secundarias. Una elección útil para prevenir y reducir la desmineralización puede ser un adhesivo antibacteriano. Objetivos: El propósito es determinar la resistencia al cizallamiento de un antibacteriano experimental, un adhesivo de autograbado y otro adhesivo, usando un sellador de fisuras y un composite sobre el esmalte intacto.
Métodos. 24 molares humanos extraídos se seccionaron vestíbulo-lingualmente para obtener dos superficies de esmalte muy planas. Se repartieron en 4 grupos 48 muestras asignadas aleatoriamente. Los grupos se prepararon para recibir los siguientes tratamientos: Grupo 1 : Admira Bond + Admira Seal (n = 10); Grupo 2 : ABF Bond + Admira Seal (n = 11); Grupo 3 : Clearfil SE Bond + Clearfil AP-X (n = 12); Grupo 4 : ABF Bond + Clearfil AP-X (n = 11). Se colocó un molde cilíndrico sobre la superficie adhesiva y ambos materiales se aplicaron según las instrucciones. Los dientes se termociclaron 500 ciclos entre 50 y 550 con un tiempo intermedio de 30 segundos. Para la prueba de resistencia al cizallamiento, la fuerza se aplicó verticalmente a la base

del molde. La velocidad media de cruceta fue de 5 mm/min.
Resultados. Se determinó la máxima fuerza que una muestra puede resistir hasta romperse y se calculó la resistencia al cizallamiento dividiendo la fuerza de fractura por el área de la superficie del molde. Los resultados fueron (mediano, mínimo, máximo, respectivamente) Grupo 1 (7,9 MPa; 4,2; 13,6), Grupo 2 (8,6 MPa; 2,6; 15,9), Grupo 3 (5,9 MPa; 5,3; 8,2) Grupo 4 (8,8 MPa; 3; 19,2). Para el análisis estadístico se realizaron las pruebas de Kruskal-Wallis y Mann-Whitney. Los grupos 3 y 4 eran estadísticamente diferentes (p = 0,005; p < 0,05). No hubo diferencias estadísticas entre los grupos sellador y composite para ABF (p = 0,375). Los sitios de fractura se vieron con microscopio 16X de magnificación para determinar si el modo de fractura es adhesivo o cohesivo. *Los resultados fueron.* (n° de muestras con fractura adhesiva, n° de muestras con fractura cohesiva) para el grupo 1 (7; 3), 2 (3, 8), 3 (2, 9), 4 (8, 3).
Conclusión. El adhesivo ABF, además de su eficacia antibacteriana, está suficientemente cualificado para ser usado bajo un sellador de fisuras y una resina composite sobre el esmalte intacto.

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