Bond Strength to Unground Enamel and Sealing Ability in Pits and Fissures of a New Self-Adhering Flowable Resin Composite

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Objective: *To evaluate the applicability as a sealant of a new self-adhering flowable resin composite (Vertise Flow, Kerr, VF) by assessing shear bond strength (SBS) to unground enamel and microleakage (µLKG) in sealed pits and fissures.* ***Study Design****: Marketed sealants to be used in combination with phosphoric acid (Guardian Seal, Kerr, GS) or with a self-etch adhesive (Adper Prompt-L-Pop/Clinpro Sealant, 3M ESPE, CS) were compared to VF. For SBS testing on unground enamel 10 molars per group were used. For µLKG assessment, pits and fissures sealing was performed in 12 molars per group. The sealed teeth were immersed in a 50% weight silver nitrate solution for 24 hours and the extent of interfacial leakage was measured. Between-group differences in SBS were assessed using One-Way Analysis of Variance (ANOVA), followed by Tukey test ($p<0.05$). µLKG data were analyzed with Kruskall-Wallis ANOVA ($p>0.05$).* ***Results****: SBS of VF was statistically similar to that measured by CS and higher than that of GS. Interfacial leakage was similar in the three groups.* ***Conclusions****: The finding of satisfactory bond strength and sealing ability of VF when compared to the marketed sealants encourages the use of VF in pit and fissure sealing.*
Keywords: *Pit and fissure sealant, All-in-one adhesives, Self-adhering flowable resin composite, Shear bond strength, Microleakage.*

INTRODUCTION

Current dentistry privileges minimal intervention methods, among which pit and fissure sealing is a widely accepted approach for preventing occlusal caries. Sealants bond micromechanically and act as physical barriers that block the flow of nutrients to the fissure microorganisms, thus making the surface beneath the sealant less susceptible to caries.[1-3]

Most commonly used systems for sealing are lightly filled or unfilled resin based materials in combination with phosphoric acid for etching.[4-7] The latter renders the enamel surface porous, while the low viscosity of the sealants allows penetration of the material into pits and fissures, forming a resin-impregnated layer of enamel.[8]

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Incorporation of a bonding agent layer between the sealants and enamel has been investigated and its beneficial effect, firstly shown by Hitt and Feigal,[9] was further confirmed by various studies.[10-13] The potential of all-in-one adhesives in sealing procedures has also been explored.[14-15] These adhesives etch, prime, and bond the dental substrates simultaneously, thus allowing to simplify the bonding procedure and reduce chair-time.[16-18] From a clinical viewpoint, such properties are particularly attractive in pediatric dentistry, where patient compliance is an issue and the achievement of proper isolation is more challenging.[19]

Recently, a new self-adhering flowable resin composite, Vertise Flow (Kerr, Orange, CA, USA), has been introduced to the dental market. According to the manufacturer, this innovative material combines the rheological properties of a flowable resin composite with the adhesive potential of a bonding agent. Therefore, the need for separate bonding application is eliminated and treatment time is minimized. Vertise Flow has been indicated for the restoration of small Class I cavities, Class V cavities, non-carious cervical lesions, for lining in Class I and Class II restorations, and for pit and fissure sealing.

Clinical and laboratory studies are required for the validation of a material. The sealing ability of Vertise Flow in Class V cavities was assessed in a recent microleakage study.[20] A 6-month clinical trial documented a satisfactory performance of Vertise Flow in Class I restorations.[21] Other studies explored properties of Vertise Flow related to water sorption.[22-23] Further research is ongoing to evaluate several proposed clinical applications of this adhesive-free restorative material. Nevertheless, no data on the potential of Vertise Flow as a pit and fissure sealant have yet been published in indexed journals. Therefore the present study was aimed at testing Vertise Flow in its bond strength to intact enamel and sealing ability of pits and fissures. For comparative purposes marketed sealants to be used

Table 1. Chemical composition and instructions for use of the tested materials.

Group	Material/Shade	Batch Number	Chemical Composition	Application
1	Etchant Gel	3036049	37.5% Phosphoric Gel	Application for 15s, rinsing for 10s, air-drying for 5s.
	Vertise Flow/A2	3172311	GPDM; Prepolymerized filler, 1-micron barium glass filler, nano-sized colloidal silica, nano-sized Ytterbium fluoride (filler load 70% wt).	Application of <0.5mm layer and brushing for 15-20s, light-curing for 20s. Application of a second layer and light-curing for 20s.
2	Etchant Gel	3036049	37.5% Phosphoric Gel	Application for 15s, insing for 10s, air-drying for 5s.
	Guardian Seal	3218533	Fluoride releasing monomer (agent BF3), Bis-GMA, CQ, silica (filler load 30% wt)	Application in a uniform layer, light-curing for 30s.
3	Adper Prompt L-Pop	311435	Liquid 1: Methacrylated phosphoric esters, Bis-GMA Initiators based on camphorquinone, Stabilizers; Liquid 2: Water, HEMA, Polyalkenoic acid, Stabilizers. pH=0.7	Application and agitation for 15s, air-drying.
	Clinpro Sealant	82T	Bis-GMA, TEGDMA, EDMAB, CQ, BHT, silica (7%), TBATFB, TiO2, rose Bengal sodium	Application and light-curing.

in combination with phosphoric acid or with a self-etch adhesive were also evaluated.

The following null hypothesis was subjected to testing: a new self-adhering flowable resin composite and two marketed sealants achieve similar shear bond strength to unground enamel and comparable seal of pits and fissure walls.

MATERIALS AND METHOD

A sample of sixty six molars was collected following informed written consent from the donors. Teeth were stored in 0.5% Chloramine T solution at 4°C for preventing bacterial growth for no longer than three months until used in the experiment.

Shear Bond Strength Tests

Thirty molars were randomly destined to shear bond strength testing. Roots were cut off 2mm below the cementum-enamel junction with a water-cooled, low-speed diamond saw (Isomet, Buehler, Lake Bluff, IL, USA). The crowns were embedded in acrylic resin with their sound buccal or lingual surface displayed. On each tooth the enamel surface was cleaned with a non-fluoride, oil-free paste (Nupro Prophylaxis Paste, Dentsply Detrey, Kostanz, Germany), applied by a brush mounted on a low-speed handpiece under water cooling. A new brush was mounted after five cleanings.

Teeth were randomly allocated to three equally sized groups (n=10), that were defined based on the sealant to be tested as follows:

Group 1 – Etchant Gel + Vertise Flow (Kerr, Orange, CA, USA);

Group 2 – Etchant Gel + Guardian Seal (Kerr, Orange, CA, USA);

Group 3 – Adper prompt L-Pop + Clinpro Sealant (3M ESPE, St. Paul, MN, USA);

In order to test the materials on a standardized bonding area, an aluminum split mold was used to hold onto the substrate a silicon mold with a 3-mm internal diameter. The enamel substrate was treated as per manufacturer's instructions (Table 1). In group 1 and 2 specimens the enamel surface was etched with 37.5% phosphoric acid, while in group 3 the self-etch adhesive Adper Prompt L-Pop was used. Vertise Flow was applied onto the enamel surface in a first 0.5-mm thick layer that was rubbed for 15-20s with the proprietary microbrush and light-cured with a quartz-tungsten-halogen curing device (VIP, Bisco Inc, Schaumburg, IL, USA; 600 mW/cm^2). Then, a second increment was layered and photopolymerized, so as to create a 2-mm thick build-up. Guardian Seal and Clinpro Sealant were applied in a 2-mm thick layer over the conditioned enamel substrate and cured using the same light source as in Group 1. The bonded specimens were then left undisturbed for 24 hours in 100% humidity at 37°C prior to shear bond strength testing. Using a universal testing machine (Triax Digital 50, Controls,132 Milan, Italy), a shear load was applied in a direction parallel to the bonded interface at a crosshead speed of 0.5mm/min until failure occurred. The load at failure was recorded in Newtons (N). The diameter of the debonded composite cylinder was measured with a digital caliper (Orteam srl, Milan, Italy). Bond strength was then calculated in MegaPascals (MPa) by dividing the load at failure by the adhesive surface area (mm^2). Failure modes were evaluated by a single operator (MM) under an optical microscope (Nikon type102, Tokyo, Japan) at 40x magnification, and classified as cohesive within the substrate (enamel or sealing material), adhesive (between sealing material and enamel) or mixed (if adhesive and cohesive fractures occurred simultaneously).

Microleakage

Thirty-six molars were used for sealing evaluation. The occlusal surface of the teeth was cleaned with a non-fluoride, oil-free paste (Nupro Prophylaxis Paste, Dentsply Detrey Kostanz, Germany), applied by a brush mounted on a low-speed handpiece under water cooling. A new brush was mounted after five cleanings.

Teeth were randomly divided into three groups (n=12) based on the material to be used for pits and fissures sealing. The groups were defined as in shear bond strength testing (Table 1). Following

Table 2. Descriptive statistics of shear bond strengths. Different letters label statistically significant differences according to the post-hoc test ($p<0.05$).

Groups	N	Mean (MPa)	Std. Deviation	Significance p<0.05
Phosphoric Acid/Vertise Flow	10	17.9	2.9	A
Adper Prompt-L-Pop + Clinpro Sealant	10	12.9	6	AB
Phosphoric Acid/Guardian Seal	10	11.7	4.6	B

enamel substrate treatment with phosphoric acid or the self-etch adhesive in Groups 1-2 and Group 3 respectively, the sealing material was applied to pits and fissures walls as it would have been done clinically. Light-curing was performed using the same device as in the preparation of shear bond strength specimens. Two randomly chosen specimens per group were kept for scanning electron microscopy observations.

After 24-hour storage at 100% humidity and 37˚C, restored teeth were covered with 2 layers of fast-setting nail varnish applied up to within 1mm of the sealed interface. Before their dehydration, teeth were immersed into 50% weight silver nitrate solution ($AgNO_3$) solution and left undisturbed for 24 hours. The silver-impregnated teeth were thoroughly washed with distilled water and placed into a photo-developing solution for 8 hours (Dental X-Ray Developer, Kodak Co, Rochester, NY, USA). The teeth were again abundantly rinsed with water, and cut into two halves with a low-speed diamond saw under water-cooling (Isomet, Buehler Ltd, 83 Lake Bluff, II, USA). The obtained sections were kept moist until the microscopic observations took place. A digital image of each section was acquired using a photo-camera (D80, Nikon Co, Tokyo, Japan) equipped with a Medical-Nikkor lens (Nikon Co, Tokyo, Japan) at 2x magnification. In order to quantify the microleakage on the digital image of each tooth half, the length of stained tooth-sealant interface was measured in pixels and related to the total interfacial length, also measured in pixels, using the image analysis software Digimizer V.3.0.0 (MedCalc Software, Mariakerke, Belgium). The percentage of microleakage was thus calculated:

(Length of stained interface/total interfacial length) x100.

The calculations were performed on both sides of each slice, but only the side exhibiting the higher leakage was considered in the statistical analysis.

Scanning Electron Microscopy

Two randomly selected specimens per group were processed for SEM observations of interfacial adaptation. A 2-mm thick slab containing the interface of interest was sectioned from each tooth and polished with wet SiC papers of increasingly finer grit (Buelher, #600, #1000, #1200). The interface was brought into relief by etching with 32% silica-free phosphoric acid gel (Uni-Etch, Bisco, Schaumburg, IL), followed by brief deproteinization with a 2% sodium hypochlorite solution for 60s. After rinsing with de-ionized water, specimens were dehydrated in an ascending series of aqueous ethanol solutions to an absolute ethanol, and dried using hexamethyldisilazine (HMDS, Carlo Erba, Rodano, Iatly). Specimens were then mounted on aluminum stubs, coated with a 15-20 nm thick layer of gold by means of the SC7620 Sputter Coater device (Polaron Range, Quorum Technologies, England), and inspected by a scanning electron microscope (JSM-6060LV, JEOL, Tokyo, Japan) at 500X and 1000X magnifications.

Statistical analysis

Shear bond strength

As the data distribution was normal according to the Kolmogorov-Smirnov test and group variances were homogenous according to the Levene test, One-Way Analysis of Variance (ANOVA) was applied, followed by the Tukey test for post hoc comparisons. In all the analyses the level of significance was set at $\alpha=0.05$.

Microleakage

As the data distribution was not normal according to the Kolmogorov-Smirnov test, the Kruskall-Wallis ANOVA on Ranks was applied in order to assess the statistical significance of between-group differences. In all the analyses the level of significance was set at $\alpha=0.05$.

RESULTS

Descriptive statistics of shear bond strengths and microleakage, along with the statistical differences of between-group differences are reported in Table 2 and Table 3 respectively. Different letters label statistically significant differences.

Shear bond strength

The One-Way ANOVA revealed that the shear bond strengths of the tested materials differed significantly ($p=0.015$, Table 2). Vertise Flow recorded the highest shear bond strength and the difference was statistically significant with Guardian Seal.

Adhesive fracture was the only occurring failure mode in all the groups.

Microleakage

The tested materials were not found to differ significantly with regard to microleakage ($p=0.07$, Table 3).

SEM

All the materials established a continuous interface with the enamel substrate (Fig. 1). However, a deeper resin penetration was seen in Vertise Flow (Fig. 1a) and Guardian Seal (Fig. 1b) specimens. A defined resin-impregnated layer of enamel was visible in Guardian Seal specimens (Fig. 1b), while Vertise Flow did not produce a distinct enamel hybrid layer. Limited etching effect was seen in

Table 3. Descriptive statistics of microleakage measurements

Groups	N	Median (%)	Interquartile range
Phosphoric Acid/Vertise Flow	10	2.2	6.3
Adper Prompt-L-Pop + Clinpro Sealant	10	0	0
Phosphoric Acid/ Guardian Seal	10	0	0.5

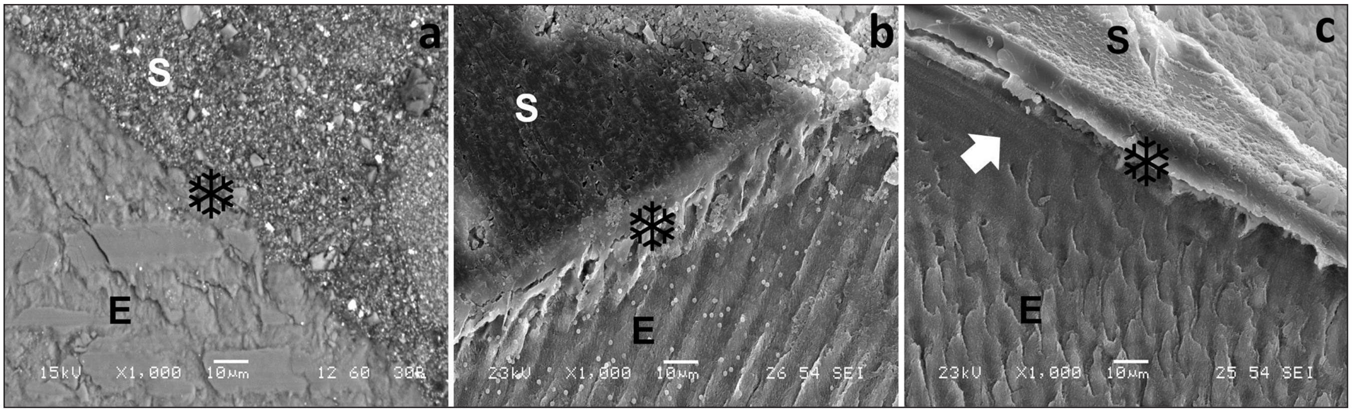

Figure 1. Representative images of Scanning Electron Microscopy of enamel-sealant interface indicated with the asterisk (magnification 1000x, bar 10 µm). (a) Vertise Flow, (b) Guardian Seal, (c) Clinpro Sealant. E: Enamel, S: Sealant. In the specimens etched with phosphoric acid (a, b) the superficial layer of aprismatic enamel was removed and the resin infiltrated the conditioned substrate. A deeper resin penetration pattern was visible in Guardian Seal specimens (b). The etching pattern of Clinpro Sealant (c) was more superficial and some aprismatic layer was retained (an arrowhead).

Clinpro Sealant specimens, where aprismatic layer was still detectable (Fig. 1c).

DISCUSSION

In the present study the use of a new self-adhesive flowable resin composite as a sealant was pre-clinically assessed by measuring shear bond strength to unground enamel and microleakage in pits and fissures. Bond strength is thought to be predictive of materials' retentive ability, based on the consideration that the higher the bond strength, the stronger the resistance to curing stress and oral function loading.[16] With reference to the sealing ability, it was assessed that the lack of optimal marginal sealing allows passage of bacteria and their accumulation on the bottom of pits and fissures[24-25] Microleakage analysis using dyes was shown to be indicative of the sealing ability[26-27] and predictive of bacteria percolation, as the size of tracer molecules conforms to that of bacteria.[27-28]

Since the self-adhering flowable resin composite Vertise Flow measured significantly higher bond strength than Guardian Seal, the formulated null hypothesis has to be rejected. Vertise Flow is a novel adhesive-free restorative system featuring the handling of a flowable composite and a self-adhering potential. The bonding mechanism of Vertise Flow relies on the bifunctional adhesive monomer Glycerol phosphate dimetracrylate (GPDM), whose acidic phosphate groups bond with calcium ions, while the methacrylate functional groups copolymerize with other methacrylate monomers, thus increasing crosslinking density and mechanical strength. Pit and fissure sealing is one of the clinical indications of Vertise Flow. Due to the presence of prismless enamel in intact pits and fissures, in order to achieve proper adhesion of Vertise Flow, the manufacturer recommends that the substrate should be preliminarily etched with phosphoric acid. In addition,15-20 s brushing of a first 0.5mm thick layer of material is requested to enable close proximity of the adhesive monomers with the substrate [Kerr technical bulletin].

The performance of Vertise Flow was assessed with reference to the outcome of two previously marketed sealants.

Clinpro Sealant is a low viscosity, fluoride releasing composite resin with an exclusive light-activated color-change technology. Clinpro Sealant was used in combination with the proprietary self-etch adhesive. The bond strength of Clinpro Sealant was lower, yet statistically comparable to that of Vertise Flow. As a possible explanation for the higher bond strength of Vertise Flow in comparison with Clinpro Sealant, the peculiar dynamics of Vertise Flow adhesion/polymerization process can be considered. When the adhesive solution and the resin-based sealant are applied consecutively, as in the case of Clinpro Sealant, curing stress of the sealant competes against the bond just established by the adhesive.[29] Conversely, with Vertise Flow bonding and polymerization take place simultaneously. It can therefore be speculated that the competition between bond strength and curing stress is reduced as the viscous-elastic flow occurs concurrently with the bonding process.

Guardian Seal is a light-cured resin, filled by 30% in weight and with fluoride release ability. Guardian Seal requires preliminary phosphoric acid-etching. The bond strengths measured by Guardian seal were significantly lower than those of Vertise Flow. Such difference could be related to the absence of any adhesive potential of Guardian Seal.

With respect to marginal adaptation, the tested materials exhibited similar effectiveness at sealing pit and fissure walls. Similarity in sealant-enamel interfacial microleakage following substrate treatment with phosphoric acid or with a self-etch adhesive was reported in a previous study.[30] Concerning the miscroscopic aspects of adhesion that were revealed by SEM observations, phosphoric acid etching in Vertise Flow (Fig. 1a) and Guardian Seal (Fig. 1b) specimens, removed aprismatic enamel that is known to be less conducive to bonding,[31-34] thus promoting resin infiltration (Fig. 1a, b). It was probably in relation to the lower filled load (Table 1) and lower viscosity that Guardian Seal penetrated more deeply than Vertise Flow into the conditioned enamel. On the other hand, Adper Prompt-L-Pop, that was used in combination with Clinpro Sealant, although classified as a strong self-etch adhesive (pH=0.7), effected a limited modification of the superficial prismless enamel (Fig. 1c). Notwithstanding the more shallow interaction, Clinpro Sealant did not prove inferior to the other materials neither in bond strength nor in the quality of the seal. As a matter of fact, no direct correspondence emerged in the present study between the outcome of shear bond strength testing, microleakage assessment and morphological observations. However, the lack of any correlation between bond strength and leakage has been reported in several previous studies.[35-37]

It has been documented that fissure anatomy influences the efficacy of a sealant. Specifically, fissures with wider occlusal length were characterized to be more susceptible to the formation of gaps, while deeper fissures are believed to allow better resin adaptation.[38] This paper was not intended to evaluate the influence of fissure anatomy on sealant performance and the authors assumed that random allocation of the teeth to the experimental groups equally distributed morphological variations.

It should also be pointed out that by measuring shear bond strength and microleakage 24 hours after the sealing procedure had been performed, the early bonding and sealing ability of the materials was assessed in the present study. Further laboratory tests involving aging procedures able to simulate the clinical function would be desirable to strengthen the collected evidence. Such studies could provide additional information on clinically relevant properties of the materials, such as wear resistance and water sorption related phenomena. It has been demonstrated that Vertise Flow undergoes hygroscopic expansion.[22-23] It can be speculated that such dimensional change may help marginal adaptation if it effectively balances polymerization shrinkage. It can also be guessed that owing to the relatively high filler load, Vertise Flow may better resist to wear in comparison with unfilled or lightly filled resin formulations for pit and fissure sealing. However, all these considerations remain at the hypothesis level until adequate in vitro protocols or, ideally, clinical trials are carried out to verify them.

CONCLUSIONS

Within the limitations of this *in vitro* study, the finding that the new self-adhering flowable resin composite Vertise Flow achieved similar bond strength to unground enamel and similar sealing ability when compared with a marketed sealant encourages the use of Vertise Flow in pit and fissure sealing. As Vertise Flow might already been in the clinic inventory for the use in direct restorations, its applicability also to pit and fissure sealing would eliminate the need of a specific product for this preventive procedure. Further in vitro studies involving aging methods, as well as clinical trials should be performed to reinforce the evidence collected in the present investigation.

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