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Bond strengths of an antibacterial monomer-containing adhesive system applied with and without acid etching for lingual retainer bonding

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SUMMARY The aim of this study was to test the null hypothesis that there is no significant difference in bond strength and failure site location of composite bonded to etched and unetched enamel with an antibacterial monomer-containing adhesive and a conventional lingual retainer adhesive system. The crowns of 60 extracted lower human incisors were mounted in acrylic resin leaving the lingual surface of the crowns parallel to the base of the moulds. The teeth were randomly divided into three equal groups: two experimental and a control. Conventional lingual retainer composite (Transbond LR) and antibacterial monomer-containing adhesive (Clearfil Protect Bond), with or without prior etching, were applied to the lingual surface of the teeth by packing the material into cylindrical plastic matrices (Ultradent) with an internal diameter of 2.34 mm and a height of 3 mm to simulate lingual retainer bonding. The shear bond data were analysed using analysis of variance and Tukey's tests. Fracture modes were analysed by chi-square test.

Statistical analysis showed that the bond strengths of the control (Transbond LR, mean: 24.77±9.25 MPa) and Clearfil Protect Bond with etching, (mean: 20.24±8.5 MPa) were significantly higher than Clearfil Protect Bond without etching, (mean: 12.56±6.93 MPa). In general, a greater percentage of the fractures were adhesive, at the tooth–composite interface (60–65 per cent). No statistically significant difference was found among the groups. The hypothesis is thus rejected. Within the limits of this *in vitro* model, antibacterial monomer-containing self-etch adhesive with acid etching did not significantly affect shear bond strength when compared with the control. However, the same adhesive used without acid etching resulted in a significant decrease in bond strength.

Introduction

As new materials and techniques are introduced, orthodontists adopt some of these innovations and add them to their routine practice (Bishara *et al.*, 2007), including the use of self-etching primers (SEPs), resin-modified glass ionomers, chlorhexidine- or fluoride-containing varnishes, and different adhesives. Acidic monomers containing SEPs eliminate the etching and rinsing application steps. It has been suggested that this improves the efficiency in clinical procedures by reducing the chair-side time.

Demineralization is a common side-effect of fixed appliance orthodontic treatment (Millett *et al.*, 1999). It occurs when the pH of the oral environment favours diffusion of calcium and phosphate ions out of enamel, and is reported to occur in 2–96 per cent of orthodontic patients (Mitchell, 1992). Årtun (1984) investigated the potential caries, demineralization, and periodontal reactions associated with long-term use of different types of bonded lingual retainers and concluded that, regardless of the type of wire involved in construction of the 3–3 retainers, there is a tendency for plaque and calculus to accumulate along the retainer wires, and this tendency seems to increase with time.

In order to inhibit the development of carious lesions in patients with fixed appliances, bacterial biofilm around the fixed appliances was used to control and maintain a constant level of fluoride in the oral cavity (Stephen, 1997; Derks *et al.*, 2004). Fluoride-releasing bonding material showed almost no demineralization-inhibiting effect (Derks *et al.*, 2004). For that reason, it has been suggested that the combined use of antimicrobials and fluoride may enhance the cariostatic effect (Buyukyilmaz and Øgaard, 1995).

Imazato *et al.* (1994, 1999) reported the achievement of an antibacterial adhesive system by incorporation of the monomer, 12-methacryloyloxydodecylpyridinium bromide (MDPB), that has strong bactericidal activity against oral bacteria. Based on the results obtained, a new single-bottled 5 per cent MDPB-containing primer was developed, and this two-step mild self-etching and fluoride-releasing adhesive system employing this primer was commercialized as Clearfil Protect Bond.

However, some concern remains regarding the bonding efficiency of self-etch adhesives to enamel, specifically when so-called 'mild' (pH around 2 or above) self-etch adhesives are used (Kanemura *et al.*, 1999; Pashley and Tay, 2001). Some manufacturers even recommend prior acid etching with phosphoric acid when bonding to enamel. The extent and the depth of the etching pattern should influence the bond strength of an adhesive.

Van Landuyt *et al.* (2006), who converted a two-step self-etch adhesive (Clearfil SE Bond) into a three-step etch-and-rinse adhesive by prior acid etching, reported that pre-treatment etching significantly increased the bonding effectiveness of Clearfil SE Bond to enamel.

Clearfil Protect Bond has been previously examined with regard to bracket bonding (Bishara *et al.*, 2005; Korbmacher *et al.*, 2006). However, no studies have been performed to investigate the bond strength of this material as orthodontic lingual retainer adhesive. Therefore, the aim of this study was to examine whether additional prior etching with phosphoric acid (37 per cent) provides any supplementary effect on the bond strength of lingual retainer composite when bonded with the antibacterial monomer containing Clearfil Protect Bond.

For the purpose of this study, the null hypothesis assumed that there were no statistically significant differences in bond strength or failure site location of composites bonded to enamel with an antibacterial monomer-containing adhesive system, with or without prior etching, and a conventional lingual retainer adhesive system.

Materials and methods

Sample preparation

Mandibular incisors extracted due to periodontal reasons were stored at +4°C in a physiological saline solution for 1 month. Teeth with hypoplastic areas, cracks, or gross irregularities of the enamel structure were excluded from the study. The criteria for tooth selection dictated no pre-treatment with a chemical agent such as alcohol, formalin, or hydrogen peroxide. Soft tissue remnants and calculus were removed from the teeth, following which they were cleaned with a fluoride-free pumice and rubber cup.

Sixty extracted teeth were selected. The roots of the teeth were cut off with a water-cooled diamond disk and the crowns mounted using chemically cured acrylic resin (Vertex, Zeist, The Netherlands) in a 3 cm diameter circular mould, with the lingual enamel surfaces exposed.

Detailed information of the products used are shown in Table 1. The teeth were distributed into three equal groups: two experimental and a control. The groups and sample preparation techniques were as follows:

Transbond LR control: All samples in this group were etched for 15 seconds with 37 per cent orthophosphoric acid (3M Dental Products, St Paul, Minnesota, USA), rinsed with water from a three-in-one syringe for 15 seconds, and dried with an oil-free source for 15 seconds. Before composite placement, Transbond XT primer (3M Unitek, Monrovia, California, USA) was applied as a thin uniform coat using a brush to the etched surfaces. The primer was cured for 10 seconds. A conventional orthodontic lingual retainer composite, Transbond LR (3M Unitek), was placed onto enamel surface by packing the material into cylindrical-shaped plastic matrices (Ultradent, South Jordan, Utah, USA; Figure 1) with an internal diameter of 2.34 mm and a height of 3 mm. Excess composite was carefully removed from the periphery of the matrix with an explorer.

Clearfil Protect Bond with prior acid etching: Twenty teeth were etched with 37 per cent orthophosphoric acid (3M Dental Products) for 15 seconds. The teeth were then washed with a water spray for 15 seconds, dried to a chalky white appearance, and the SEP containing the antibacterial monomer Clearfil Protect Bond (Kuraray Medical Inc., Okayama, Japan) was applied using a brush to the etched surface for 20 seconds, and sprayed with a mild airstream to evaporate the solvent. Clearfil Protect Bond was then applied, gently air flowed, and light cured for 10 seconds. After these steps, Transbond LR composite was applied by packing into cylindrical-shaped plastic matrices and cured.

Clearfil Protect Bond without prior acid etching: Clearfil Protect Bond SEP was applied as recommended by the manufacturer. The enamel was wiped with primer

Table 1 Materials used and chemical compositions.

Material	Manufacturer	Component	Chemical composition
Orthophosphoric Acid	3M Unitek	Etching gel	37% phosphoric acid
Transbond XT™	3M Unitek	Primer	Triethylene glycol dimethacrylate, bisphenol A diglycidyl ether dimethacrylate
Clearfil Protect Bond	Kuraray Medical Inc.	Primer	MDP, MDPB, HEMA, hydrophilic dimethacrylate, water, initiators
Clearfil Protect Bond	Kuraray Medical Inc.	Bond	MDP, HEMA, Bis-GMA, hydrophobic dimethacrylate, DL-camphorquinone, *N,N*-diethanol-*p*-toluidine, silanated colloidal silica, surface-treated sodium fluoride
Transbond™ LR	3M Unitek	Paste	Bisphenol a diglycidyl ether dimethacrylate, triethylene glycol dimethacrylate, dichlorodimethylsilane reaction product with silica

MDP, 10-methacryloyloxydecyldihydroges phosphate; MDPB, methacryloxydodecylpyridinium bromide; HEMA, 2-hydroxyethylmethacrylate; Bis-GMA, bisphenol A digliycidylmethacrylate.

Figure 1 Device used for placing standard composite blocks on enamel surface.

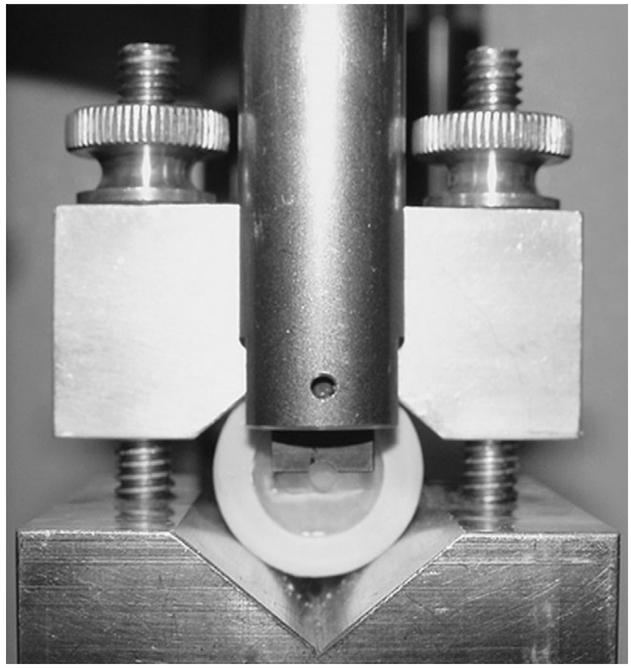

Figure 2 Application of force on the composite block with a notch-shaped apparatus.

for 20 seconds and dried with a mild airflow. Clearfil Protect Bond was then applied, gently air flowed, and light cured for 10 seconds. After these steps, similar composite placement was performed as in groups 2 and 3.

A quartz tungsten halogen light unit (Hilux 350, Express Dental Products, Toronto, Canada) with a 10 mm diameter light tip was used for curing the adhesive resin and composite in all groups. Transbond LR composite was cured for 20 seconds. The specimens were then stored in distilled water at 37°C for 24 hours before bond strength testing.

Debonding procedure

For shear bond testing, the specimens were mounted in a universal testing machine (Hounsfield Test Equipment, Salford, Lancashire, UK). A notch-shaped apparatus (Ultradent) attached to a compression load cell at a cross-head speed of 0.5 mm/minute was applied to each specimen at the interface between the tooth and composite until failure occurred (Figure 2). The maximum load (*N*) was divided by the cross-sectional area of the bonded composite posts to determine bond strength in megapascals (MPa).

Fracture analysis

Fracture analysis was performed using an optical stereomicroscope (×20 magnifications; SZ 40, Olympus, Tokyo, Japan). Failures were classified as cohesive if more than 80 per cent of the resin remained on the tooth surface, adhesive if less than 20 per cent of the resin remained on the tooth surfaces, or mixed if certain areas exhibited cohesive fractures and others adhesive fractures.

Statistical analysis

All statistical analyses were performed with the Statistical Package for Social Sciences (SPSS for Windows 13.0, SPSS Inc., Chicago, Illinois, USA). Descriptive statistics, including the mean, standard deviation, minimum, and maximum values, were calculated for the three groups. The Shapiro–Wilks normality test and Levene's variance homogeneity test were applied to the bond strength data. The data showed normal distribution, and there was homogeneity of variances between the groups. Comparisons of the mean of the shear bond strength (SBS) values were carried out with one-way analysis of variance (ANOVA). Multiple comparisons were undertaken using Tukey's honestly significant difference (HSD) test. The chi-square test was used to determine significant differences in the fracture modes among the three groups. Significance was pre-determined at $P<0.05$.

Results

The descriptive statistics for each group are presented in Table 2. The results of the ANOVA revealed statistically significant differences in bond strength among the three groups ($F=45.579$, $P=0.000$). Thus, the null hypothesis of this study was rejected. The Tukey's HSD test showed that the bond strengths of group 1 (mean: 24.77±9.25 MPa) and group 2 (mean: 20.24±8.50 MPa) were significantly higher than group 3 (mean: 12.56±6.93 MPa).

The fracture patterns for the various groups tested are listed in Table 3. The results of the chi-square comparisons indicated that there were no significant differences among the three groups ($\chi^2=2.145$, $P=0.709$). In general, a greater percentage of the fractures were adhesive at the

Table 2 Descriptive statistics and results of analysis of variance (ANOVA), comparing the bond strength of the three groups tested.

Groups tested	*n*	Bond strength (MPa)				ANOVA (F=45.579)	Test*
		Mean	Standard deviation	Minimum	Maximum		
Transbond LR	20	24.77	9.25	20.44	29.10	***P<0.001	A
Clearfil Protect Bond with prior acid etching	20	20.24	8.50	16.25	24.22		A
Clearfil Protect Bond without prior acid etching	20	12.56	6.93	9.31	15.80		B

*Groups with different letters are significantly different from each other.

Table 3 Modes of failure after shear bond testing.

Groups tested	*n*	Failures			Chi-square value	Significance
		Adhesive (%)	Cohesive (%)	Mixed (%)		
Transbond LR	20	12 (60)	1 (5)	7 (35)	2.140	NS, P=0.709
Clearfil Protect Bond with prior acid etching	20	13 (65)	0 (0)	7 (35)		
Clearfil Protect Bond without prior acid etching	20	12 (60)	0 (0)	8 (40)		

NS, not significant.

tooth–composite interface (60 per cent in group 1, 65 per cent in group 2, and 60 per cent in group 3).

Discussion

Caries prevention in orthodontic patients is of concern (Zimmer and Rottwinkel, 2004). The application of fluoride is one approach to reduce the caries risk. Various fluoride procedures have been demonstrated as effective non-invasive methods by which to inhibit the demineralization and promote the re-mineralization of enamel (Anusavice, 1997; Featherstone, 2000). However, because of the acidic environment around fixed appliances, the re-mineralization progress is hindered and more fluoride will not necessarily give a cariostatic effect (Zimmer and Rottwinkel, 2004). The combination of antimicrobial and fluoridation may effectively reduce the formation of new lesions (Øgaard *et al.*, 2001). The self-etching adhesive system, Clearfil Protect Bond, employing antibacterial primer is claimed to release fluoride. MDPB can polymerize and be immobilized within the polymer, and the bonding interface of Clearfil Protect Bond is considered to be maintained even after long-term clinical use (Imazato *et al.*, 2006). Furthermore, cured primer incorporating MDPB exhibits inhibition of bacterial growth on its surface by immobilized antibacterial components (Imazato *et al.*, 1998). It is, therefore, expected that Clearfil Protect Bond would be effective in inhibiting invading bacteria through gaps at the bonding interface after composite resin placement, leading to inhibition of caries. Thus, bonding lingual retainers to enamel with Clearfil Protect Bond is claimed to decrease the demineralization lesions under and/or around the lingual retainer where there is a high susceptibility to caries or demineralization.

However, Clearfil Protect Bond is a mild SEP with a pH value of 2.0, and the use of mild self-etch adhesives on enamel has raised some concern (Kanemura *et al.*, 1999; Pashley and Tay, 2001). The shallower etching pattern on enamel and subsequent reduced micromechanical retention might result in lower bond strength (Miyazaki *et al.*, 2000). In comparison with conventional phosphoric etching, none of the self-etching materials proved capable of achieving the etching effect of phosphoric acid (Holzmeier *et al.*, 2008). Buyukyilmaz *et al.* (2003) tested various SEPs on non-abraded enamel and concluded that Transbond™ Plus SE Primer and Clearfil™ SE Bond create an obviously porous structure on aprismatic enamel. Scanning electron microscopic image analysis should consider that SEPs are rinsed off the enamel with acetone after the prescribed contact time and that all primer components or remnants of the dissolved calcium are not always removed, meaning that the ultimate shallow etching pattern can only be recognized with some difficulty (Cal-Neto and Miguel, 2006). In this study, whether an additional prior etching with phosphoric acid (37 per cent) provides any supplementary effect on the bond strength of lingual retainer composite when bonded with the antibacterial monomer containing Clearfil Protect Bond by a SBS methodology was tested.

In the present investigation, small composite blocks were used to evaluate bond strength values between enamel and composite for simulation of the failure of lingual retainer. As composite resins were applied to the enamel surfaces in standard cylindrical-shaped plastic matrices, the area of bonding, the thickness of the composite resin, and the distance of the curing light was standardized. Subsequently, shear bond testing was performed with a notch-shaped apparatus instead of a knife-edge apparatus which wraps around the composite material, thereby contacting a larger area of the composite sample and distributing the stress over a larger surface area. In contrast to the Ultradent testing device, the knife-edge exerts the load on a smaller and more concentrated area of the bonded sample (Van Noort *et al.*, 1989; DeHoff *et al.*, 1995). With a knife-edge, premature failure of the teeth-adhesive bond may occur and this may result in lower or even incorrect SBS results (Pecora *et al.*, 2002).

SBS testing clearly indicated that phosphoric acid etching prior to Clearfil Protect Bond application increased the bond strength to enamel of lingual retainer composite when compared with the group without prior etching. The results of this research are parallel to the studies of several other authors, who also reported that etching before the application of mild-self-etch adhesive increased bond strength to enamel (Torii *et al.*, 2002; Erhardt *et al.*, 2004). Recently, Van Landuyt *et al.* (2006) evaluated the effect of prior etching with phosphoric acid on the bond strength of a two-step self-etch adhesive Clearfil SE Bond with a pH of 2. Those authors reported that pre-treatment etching significantly increased the bonding effectiveness of Clearfil SE Bond to enamel. The bond strength of Clearfil Protect Bond with prior acid etching was similar to the Transbond LR control group. However, the bond strength of Clearfil Protect Bond without prior acid etching was lower than in the control group. In order to obtain similar enamel bond strengths to Transbond XT primer which is used to bond lingual retainer composite to enamel, prior to antibacterial monomer-containing adhesive application, the enamel surface should be etched.

Reynolds (1975) determined the minimum bond strength values in direct orthodontic bonding systems that are clinically acceptable to be 5.9–7.8 MPa. The bond strength values in three groups in the present study compared favourably with those recommendations. However, clinical conditions may differ significantly from an *in vitro* setting. It needs to be emphasized that as this was a laboratory study, the test conditions were not subject to the rigours of the oral environment (Bishara *et al.*, 1998). Heat and humidity conditions in the oral cavity are highly variable. Because of the probable differences between *in vivo* and *in vitro* conditions, as well as the testing method, a direct comparison cannot be made with the findings of other studies.

Most orthodontic bonding studies have shown a mix or cohesive type failure (Årtun and Bergland, 1984; Oliver, 1988). In those studies, after bond strength testing some of the composite resin remained on either the enamel surface or the bracket base, causing cohesive failure rather than adhesive failure between the enamel and the composite resin. Because brackets were not used in the present study, more adhesive failures occurred and the actual bond strength between the enamel and the composite could be measured.

Conclusions

Within the limitations of this *in vitro* study, it was found that the use of an antibacterial monomer containing self-etch adhesive system with prior acid etching did not significantly affect SBS. Antibacterial monomer containing self-etch adhesive without prior acid etching resulted in a decrease in bond strength. There was no evidence to suggest a statistical difference between the failure characteristics of the groups. These results need to be confirmed clinically.

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