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Evaluation of the anti-cariogenic potential and bond strength to enamel of different fluoridated materials used for bracket bonding

Avaliação do potencial anti-cariogênico e da resistência adesiva ao esmalte de diferentes materiais contendo fluoreto, utilizados para colagem de braquetes

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Resumo

Objetivo: Avaliar *in vitro* e *in situ* o potencial anti-cariogênico e a resistência adesiva ao esmalte de materiais contendo fluoreto utilizados para a colagem de braquetes: Transbond XT (GT, controle negativo), Transbond Plus Color Change (GTF), Transbond Self Etching Primer (GSAF) and Vitremer (GV, controle positivo). **Material e método:** No estudo *in vitro*, as unidades experimentais foram premolares com braquetes colados (n = 12/grupo). Após ciclagem de pH, a liberação de F, resistência adesiva, modo de fratura e presença de mancha branca foram avaliados. No estudo *in situ*, as unidades experimentais foram fragmentos de esmalte com braquetes colados (n = 12/grupo). Os doze voluntários utilizaram dispositivos palatinos em 4 fases, com desafio cariogênico. Resistência adesiva, modo de fratura e variação de dureza superficial (%SH) foram determinados. **Resultado:** No estudo *in vitro*, a libertação de F (ppm) foi: GT=0,257±0,068c; GTF=0,634±0,100b; GSAF=0,630±0,067b; GV=2,796±1,414a. Apenas GV não apresentou lesões de mancha branca. Os valores de resistência de união (MPa) foram GT=7,62±7,18a; GTF=5,15±6,91ab; GSAF=3,42±2,97bc; GV=2,87±2,09c. A fratura adesiva foi mais frequente, com exceção de GTF. Para o estudo *in situ*, % SH foi: GT=-56,0±18,3a; GTF=-57,6±11,9a; GSAF=-57,1±11,3a; GV=-52,4±25,8a. Os valores de resistência de união foram GT = 9,5 ± 4,4a; GTF = 11,1 ± 5,9a; GSAF = 13,2 ± 6,6a; GV = 6,6 ± 4,0a. Fratura coesiva no material foi a mais frequente, exceto para GTF. **Conclusão:** Vitremer (GV) apresentou maior potencial anti-cariogênico no estudo *in vitro*, mas isso não se confirmou no estudo *in situ*. Os valores de resistência de união, a partir do estudo *in situ*, demonstraram que eles são adequados para a prática clínica.

Descritores: Resistência de união; colagem de braquetes; fluoreto; cárie dentária.

Abstract

Objective: To evaluate the *in vitro* and *in situ* anti-cariogenic potential and bond strength to enamel of materials containing fluoride (F), used for bracket bonding: Transbond XT (GT, negative control), Transbond Plus Color Change (GTF), Transbond-Self-Etching Primer (GSAF) and Vitremer (GV, positive control). **Material and method:** In the *in vitro* study, the specimens were premolars with bonded brackets (n=12/group). After pH cycling, the F release, bond strength, fracture mode and presence of white spot lesions were assessed. In the *in situ* study, the specimens were enamel fragments with bonded brackets (n=12/group). Twelve volunteers wore palatal appliances in 4 phases, with cariogenic challenge. Bond strength, fracture mode and change in surface hardness (%SH) were determined. **Result:** Relative to the *in vitro* study, F release (ppm) was: GT=0.257±0.068c; GTF=0.634±0.100b; GSAF=0.630±0.067b; GV=2.796±1.414a. Only GV showed no white spot lesions. Bond strength values (MPa) were GT=7.62±7.18a; GTF=5.15±6.91ab; GSAF=3.42±2.97bc; GV=2.87±2.09c. Adhesive fracture was the most frequent type, except for GTF. In the *in situ* study, %SH was: GT=-56.0±18.3a; GTF=-57.6±11.9a; GSAF=-57.1±11.3a; GV=-52.4±25.8a. Bond strength values were GT=9.5±4.4a; GTF=11.1±5.9a; GSAF=13.2± 6.6a; GV=6.6±4.0a. Cohesive fracture in material was the most frequent type, except for GTF. **Conclusion:** Vitremer (GV) showed the highest anti-cariogenic potential in the *in vitro* study. However, it was not confirmed by the *in situ* study. Regarding bond strength values from the *in situ* study, all materials were shown to be adequate for clinical practice.

Descriptors: Bond strength; bracket bonding; fluoride; dental caries.

INTRODUCTION

Nowadays, the prevention of dental caries is a primary objective in all areas of Dentistry. In Orthodontics, white spot lesions must be avoided, so new materials are being evaluated for this purpose[1,2].

Among the wide variety of successful preventive or therapeutic agents that have made a significant impact on people's health and quality of life, it is perhaps difficult to find one that rivals fluoride (F). The study of fluoride-releasing materials has produced good results, showing that it is capable of controlling the development of caries, whether by inhibiting demineralization or by triggering enamel/dentin remineralization[3].

Considering that the use of F during orthodontic treatment is important for protecting the enamel adjacent to the brackets[4], resin materials containing F (in the adhesive system or in the cement) have been launched for bonding brackets. Studies have been conducted to evaluate the actual anti-cariogenic potential of these materials to determine if they can be used as an alternative to resin-modified glass ionomer cements[5-9].

Studies that evaluated the F release[7] of these materials[9] have demonstrated their anti-cariogenic potential and effect on enamel demineralization around orthodontic brackets[5,6,8], as it is desirable to study these two aspects together[8].

Another important factor to evaluate is their bond strength values, which must be sufficient to support the different types of stress induced by the archwires and chewing forces; however, they should simultaneously allow the brackets to be removed from the teeth, without causing structural damage to the enamel. Bond strength values between 6 and 8 megapascals (MPa) are considered adequate for orthodontic treatment[10].

Therefore, the aim of the present study was to evaluate both the *in vitro* and *in situ* anticariogenic potential and bond strength to enamel of different F-containing materials for bonding brackets.

MATERIAL AND METHOD

The experimental groups, materials and their composition are described in Chart 1.

Ethical Aspects

Both studies (*in vitro* and *in situ*) were approved by the local Research Ethics Committee (protocols 2008/0378 and 2009/0232, respectively).

In vitro Study

This *in vitro* experiment was based on the study of Chin et al.[8]; sample size = 10/group. Brackets were bonded to 48 extracted human premolars and assigned to four experimental groups of 12 teeth each. The parameters evaluated were F release (ppm, quantitative), shear bond strength (MPa, quantitative), fracture mode (qualitative) and presence of white spot lesions (qualitative).

After extraction, all teeth were stored in 0.1% thymol. Subsequently each tooth was cleaned with pumice (S.S. White) for 10 seconds. To delineate the bonding area, two molds of adhesive tape (3×4 mm and 1×4 mm) were attached to the vestibular surfaces of all the teeth. All the test specimens were then painted with nail varnish. After two hours, the varnish had dried and the 3×4 mm strip of adhesive tape was removed. Brackets (GAC, Edgewise; 3×4 mm) were bonded to this area, in accordance with the manufacturer's instructions:

- **GT (Transbond™ XT, negative control):** the demarcated enamel surface area was acid-etched (37% phosphoric acid; FGM, Joinville) for 15 seconds, followed by 15 seconds of spraying with water and 15 seconds of drying with air from a triple syringe. The adhesive was applied with a disposable brush (FGM, Joinville) for 3 seconds and this coat was then light polymerized for 10 seconds. Resin cement was added to

Chart 1. Materials used for bracket bonding and their composition

Experimental group	Materials	Composition
GT (negative control)	Resinous cement + conventional adhesive system **(Transbond™ XT, 3M Unitek)** Batch: 0822500606	Cement: Silica, Bis-GMA, Silane, N-dimethyl benzocaine, hexafluorophosphate
		Adhesive system: triethylene glycol dimethacrylate, Bis-GMA
GTF	Resinous cement with F + conventional adhesive system **(Transbond™ Plus Color Change, 3M Unitek)** Batch: 0827600328	Cement: Bisphenol A diglycidyl ether dimethacrylate, silane treated with quartz, silane treated with silica, polyethylene glycol dimethacrylate, glass with hydrolyzed silane and oligomer of citric acid dimethacrylate.
		Adhesive system: triethylene glycol dimethacrylate, Bis-GMA
GSAF	Resinous cement + self-etching adhesive system with F **(Transbond™ Plus Self Etching Primer, 3M Unitek)** Batch: 320559c	Cement: Silica, Bis-GMA, Silane, N-dimethyl benzocaine, hexafluorophosphate
		Adhesive system: Mono- and di-hema-phosphate, camphorquinone, distilled water, aminobenzoate, potassium hexafluorotitanate, butyl hydroxy toluene, methylparaben and propylparaben
GV (positive control)	Resin-modified glass ionomer cement **(Vitremer™, 3M ESPE)** Batch: 0812000085	Powder: Fluoro-aluminosilicate glass
		Liquid: polycarboxylic acid, water, hydroxyethylmethacrylate

the bracket base before the bracket was placed on the tooth with holding tweezers, and applying slight pressure. Excess cement was removed with an exploratory probe then the material was light-polymerized for 40 seconds (10 seconds on each side; Ultralux, Dabi Atlante, Indústria Médico Odontológica).

- **GTF (Transbond™ Plus Color Change):** same procedure as for GT.
- **GSAF (Transbond™ Plus Self Etching Primer):** similar procedure as used for GT and GTF, except that acid etching and adhesive application were substituted by Transbond Self Etching Primer application with a disposable brush (FGM, Joinville), for 3 seconds, followed by a light air jet.
- **GV (Vitremer™, positive control):** one spoonful of powder and one full drop of the corresponding liquid were mixed on a block, with the use of a cement spatula for an average of 45 seconds. This mixture was spread over the entire bracket base before the bracket was placed on the tooth with holding tweezers, and applying slight pressure. Excess cement was removed with an exploratory probe then the material was light-polymerized for 40 seconds (10 seconds on each side; Ultralux, Dabi Atlante, Indústria Médico Odontológica).

One hour after bonding, all specimens were pH cycled at 37°C for 14 days, to simulate intraoral conditions. They were immersed in a (Demineralizing) acid solution for 6 hours (pH 4.3; Ca 2.0 mM; P 2.0 mM; acetate buffer 0.075M) and a (Remineralizing) neutral solution for 18 hours (pH 7.0; Ca 1.5 mM; P 0.9 mM; KCl 0.15 M; TRIS buffer 0.02M)[11]. The solutions were changed daily, and the samples from days 1, 2, 4, 7, 9 and 11 (De and Remineralizing) were stored for F analysis.

To determine the F concentration in De- and Remineralizing solutions, a F electrode (Orion 9609, Orion Research Inc. USA) coupled to a potentiometer (RbPH – 210, MSTecnopon, Brazil) was used. The electrode was previously calibrated with solutions containing 0.05 to 3.2 ppm F. All samples were buffered with TISAB III® (1:10) and the analysis was performed in duplicate. The F concentration in De- and Remineralizing solutions was added to obtain total F release at days 1, 2, 4, 7, 9 and 11.

For bond strength evaluation, the roots of each tooth were embedded in a 2.0 × 2.5 cm PVC tube with chemically activated acrylic resin (Jet Clássico). The shear bond strength test was performed with a universal testing machine (EMIC Equipamentos e Sistemas de Ensaio) with a 200 kgf load cell at a crosshead speed of 0.5 mm/min. The shear strength was calculated in kgf/cm^2 using the formula: R = F/A, where R = shear strength, F = load required to rupture the bond between bracket and tooth, and A = area of bracket base (0.1125 cm^2). The shear strength in kgf/cm^2 was transformed into MPa by multiplying the individual values by 0.0980665. After fracture, the enamel and bracket surfaces were evaluated to determine the fracture mode. The occurrence of adhesive failure was considered when the bonding material detached from the bracket base or tooth surface; cohesive failure, when the fracture occurred within the bonding material, bracket or tooth surface; mixed failure, when adhesive and cohesive failures occurred in the same specimen[12].

To evaluate the formation of artificial caries-like lesion, it was used a score according to an ordinal scale ranked from 0 to 3, by way of visual examination[11]: 0 – no caries-like lesion; 1 – Incipient or arrested caries-like lesion; 2 – Moderate active caries-like lesion; 3 – Advanced active caries-like lesion.

The fracture mode and caries-like lesions were visually analyzed in an independent and blind manner by two examiners, using a stereoscopic lens (EK3ST, Eikonal Equipamentos Ópticos e Analíticos) at 20× magnification. The results were compared, and in the event of disagreement, they jointly re-evaluated the samples until a consensus was reached.

In situ Study

In a crossover and double-blind study, conducted in four experimental phases, 12 volunteers wore a palatal appliance containing one slab of bovine enamel onto whose surface one bracket was bonded with one of the four studied materials. The parameters evaluated were: shear bond strength (MPa, quantitative); fracture mode (qualitative), and change in surface hardness (%SH, quantitative).

Thirty bovine incisors were cleaned and stored in 0.1% thymol. Subsequently, forty-eight 5.5×5.0mm slabs were obtained from the vestibular surface of the crowns of these teeth. The slabs were embedded in epoxy resin (Maxi Rubber, Diadema, SP, Brazil) using PVC molds, 2 cm in diameter, leaving the external enamel surfaces uncovered by the resin. After 24 h, the slabs were removed from the molds and flattened to obtain the smooth surfaces required for the microhardness tests. The enamel slabs were ground wet in a mechanical grinding machine (Aropol 2V, Arotec S/A Ind. Com., Cotia, SP, Brazil) with aluminum oxide discs of sequentially decreasing grain-size- (400, 600 and 1200 grit) and polished with 6, 3, ½ and ¼ mm diamond pastes (Diamond Suspension, Arotec Ind. e Comércio, São Paulo, SP, Brazil) and felt discs cooled with mineral oil, in order to obtain flat, smooth surfaces. Between the disc granulations and paste applications, the specimens were washed with distilled, deionized water.

Surface hardness measurements were performed before the bracket bonding and after the experimental phase. Before the bracket bonding, three indentations were made on each slab for each time period, spaced 100 µm apart, and assessed quantitatively according to the Knoop Hardness Number (KHN), by means of a 25 g load applied for 5s per indentation. After the experimental phases, indentations were made 100, 200 and 300 µm distant from the bracket bases.

After initial surface hardness determinations, the slabs were sterilized in moist heat (7 minutes at 130ºC and 30 min of drying at 117ºC) and kept moist in an oven at 37ºC until the brackets were bonded. They were randomly assigned to four groups (n=12/ group) according to the material used for the bracket bonding (GT, GTF, GTSAF or GV).

To delineate the area of bracket bonding and around enamel, a mold of adhesive tape (4 × 5 mm) was attached to the slab. All the slabs were then painted with nail varnish. After two hours, the varnish had dried, and the adhesive tape was removed. Brackets (GAC, Edgewise, Bohemia, NY, USA; 3 × 4 mm) were bonded to this area, in accordance with the manufacturer's instructions for each material, as previously described. Then, the slabs with the brackets were fixed on to the palatal appliances (one slab/ appliance/ experimental phase).

Twelve volunteers aged between 18 and 40 were selected. The sample size was based on the study of Gameiro et al.[13], which suggested that an *in situ* study with 10 volunteers would be sufficient to study demineralization of enamel around brackets. The volunteers were informed about the objectives of the study and signed a written term of consent to participate. The inclusion criteria for participation in the experiment were good oral hygiene conditions, evaluated via the visible plaque index. Exclusion criteria were pregnancy, those at risk of bacterial endocarditis and the presence of active caries lesions. Impressions of the maxillary dental arch of the volunteers were taken with alginate (Jeltrate Orthodontic, Dentsply, Petrópolis, RJ, Brazil) to obtain plaster casts (Durone type IV, Dentsply, Petrópolis, RJ, Brazil) for fabricating the acrylic palatal devices with chemically activated resin (JET Classic Brazilian Industry, Clean Field, SP, Brazil). The devices had one cavity into which the enamel blocks were fixed by using sticky wax. A plastic mesh was fixed over the blocks to allow biofilm accumulation.

During the experimental phases, the volunteers used the palatal devices for five consecutive days, throughout the day, except when eating. This period of five days was determined by a pilot study with four volunteers. The cariogenic challenge consisted of dropping 20% sucrose solution on to each slab, 8×/day, extra-orally. There was a wash-out period of two days between experimental phases. One week before, during (run-in) and between (wash-out) the experimental phases, the volunteers used non-fluoridated toothpaste.

To evaluate shear bond strength and fracture type analysis, after the intraoral appliances had been used, the blocks were removed and embedded in chemically-activated acrylic resin (JET, Clássico Indústria Brasileira, Campo Limpo Paulista, SP, Brazil) within a PVC matrix (Tigre, Joinville, SC, Brazil) to obtain the specimens. These were placed on the universal testing machine (EMIC - Equipamentos e Sistemas de Ensaio Ltda., São José dos Pinhais, PR, Brazil) with a chisel-type tip at a speed of 0.5 mm/min and a load cell of 200 Kgf, until the bracket-enamel bond ruptured. The data were obtained in kgf and converted to MPa according to the area of the bracket the bonding to the enamel (12 mm^2). The fracture pattern was evaluated by using a stereoscopic lens (EK3ST, Eikonal Equip. Ópticos e Analíticos, São Paulo, SP, Brazil) at 20× magnification, following the same criteria described for the *in vitro* study.

Statistical Analysis

Exploratory data analysis was performed for all response variables, for both studies. For quantitative data with homogeneity of variance, the ANOVA analysis of variance was applied. For quantitative data without homogeneity of variance and also for qualitative data, the Kruskal-Wallis test was used. A level of significance of 5% was adopted, and the statistical program SAS (SAS Institute Inc., Cary, NC, USA, Release 9.2, 2008) was used.

RESULT

Anti-cariogenic Potential

Table 1 shows the F release data in the De- and Remineralizing solutions, during days 1, 2, 4, 7, 9 and 11 of the pH cycling. The F concentration in De- and Remineralizing solutions, for the six days, were added together to obtain the total F release. The groups GTF and GSAF exhibited a similar F release, statistically different from groups GT and GV; and group GV released more F than the other groups in the study while group GT released the least amount of F.

Table 2 demonstrates the anti-cariogenic potential of the materials evaluated. In the *in vitro* study, the material with the greatest anti-cariogenic potential was GV, since it showed the highest level of inhibition of white spot formation around the brackets. As regards the *in situ* study, there was no difference in the anti-cariogenic potential among the materials evaluated. It should be stated that as there was no difference between the final mean hardness values at the different distances ($p > 0.05$), a final mean hardness value was obtained without regard to distance. There was a reduction in mean hardness values after the *in situ* phase, irrespective of treatment ($p \leq 0.05$).

Shear Bond Strength

In the *in vitro* study, statistically significant differences were found between the materials tested: Group GT produced the highest bond strength value (Table 3); Groups GTF, GSAF and GV showed average values that were inconsistent with orthodontic mechanics (lower than 6 MPa). These differences were not found in the *in situ* study, in which all the materials had bond strength values consistent with orthodontic mechanics.

Table 4 presents the frequency distribution of fracture type considering the materials used in each experimental group, in both the *in vitro* and *in situ* studies.

In the *in vitro* study, there was a higher frequency of adhesive fractures for all groups, except for GTF, which had more mixed-type fractures. Whereas, in the *in situ* study, there was a predominance of cohesive type fractures in the adhesive material, with the exception of group GTF that exhibited a higher number of mixed-type fractures.

Table 1. Median ± interquartile range (minimum to maximum) of total F release (De + Remineralizing solutions) during days 1, 2, 4, 7, 9 and 11 of pH cycling

Group	F release (ppm)	*
GT	0.257 ± 0.068 (0.168 to 0.332)	c
GTF	0.634 ± 0.100 (0.534 to 0.876)	b
GSAF	0.630 ± 0.067 (0.526 to 0.774)	b
GV	2.796 ± 1.414 (1.836 to 4.076)	a

* Distinct letters show statistically significant differences (Kruskal-Wallis, P < 0.05).

Table 2. Effect on enamel demineralization around the brackets, for the *in vitro* study (absolute and relative frequency of white spot lesions score) and *in situ* study (mean ± standard deviation of surface hardness, KHN)

group	*in vitro* study - white spot score					*in situ* study - Surface Hardness (KHN)**		
	0	1	2	3	Median*	baseline	final	%SH
GT	0 (0)	4 (33.3)	7 (58.3)	1 (8.3)	2	256.0 ± 69.6 Aa	107.3 ± 39.0 Ba	- 56.0 ± 18.3
GTF	1 (8.3)	5 (41.7)	3 (25.0)	3 (25.0)	1.5	274.8 ± 66.4 Aa	113.0 ± 30.0 Ba	- 57.6 ± 11.9
GSAF	0 (0)	5 (41.7)	2 (16.7)	3 (25.0)	2	287.0 ± 51.3 Aa	121.9 ± 37.9 Ba	- 57.1 ± 11.3
GV	12 (100)	0 (0)	0 (0)	0 (0)	0*	257.4 ± 70.0 Aa	113.3 ± 39.1 Ba	- 52.4 ± 25.8

* Statistically significant difference from the other groups (Kruskal-Wallis, $p < 0.01$); ** In the *in situ* study, values followed by different letters (uppercase on the horizontal axis and lowercase on the vertical axis) differ from one another (ANOVA; $p \leq 0.05$).

Table 3. Shear bond strength values (MPa) for the experimental groups of the *in vitro* and the *in situ* studies

Group	*in vitro**	*in situ***
GT	7.62 ± 7.18 ^{a}	9.5 ± 4.4 ^{a}
GTF	5.15 ± 6.91 ^{ab}	11.1 ± 5.9 ^{a}
GSAF	3.42 ± 2.97 ^{bc}	13.2 ± 6.6 ^{a}
GV	2.87 ± 2.09 ^{c}	6.6 ± 4.0 ^{a}

* Median ± interquartile range values; distinct letters show statistically significant differences (Kruskal-Wallis + Dunn´s test; $P < 0.05$); ** Mean ± standard deviation values; there was no statistically significant difference among the groups (ANOVA; $P > 0.05$).

Table 4. Absolute (n) and percentage (%) frequency distributions of the fracture types between the groups in the *in vitro* and *in situ* studies

	in vitro				*in situ*			
	Adhesive	Cohesive in enamel	Cohesive in material	mixed	Adhesive	Cohesive in enamel	Cohesive in material	mixed
GT	5 (41.7)	4 (33.3)	1 (8.3)	2 (16.7)	3 (27.3)	0 (0.0)	5 (45.4)	3 (27.3)
GTF	4 (33.3)	2 (16.7)	0 (0.0)	6 (50.0)	1 (10.0)	0 (0.0)	4 (40.0)	5 (50.0)
GSAF	9 (75.0)	1 (8.3)	0 (0.0)	2 (16.7)	2 (18.2)	3 (27.3)	4 (36.4)	2 (18.2)
GV	11 (100)	0 (0.0)	0 (0.0)	0 (0.0)	2 (18.2)	0 (0.0)	7 (63.6)	2 (18.2)

DISCUSSION

The present study aimed to evaluate these materials in terms of their anti-cariogenic potential and effectiveness in bonding brackets, in both controlled conditions (*in vitro* studies) and in more clinically relevant conditions (*in situ* study).

In order to evaluate the anti-cariogenic potential during the *in vitro* study, an artificial cariogenic challenge was conducted, which demonstrated a correlation with the onset and the progression of the carious lesion in vivo. According to the author, the 6 hours a day of acid challenge, by immersion of the specimens in a demineralizing solution, simulated a situation of high frequency of sucrose ingestion. On the other hand, the 18 hours in a remineralizing solution were based on the periods of repair afforded by saliva, in vivo. The pH cycling was carried out for 14 days, and the solutions at days 1, 2, 4, 7, 9 and 11 were collected for F analysis. The F release results obtained (GV > GTF and GSAF > GT) were expected values, in line with those in the literature[7-9,14].

One aspect that was not evaluated in the present study, and which should be considered was that even when no significant amounts of F are released, resin cements may act as an F reservoir resulting from the use of F dentifrices and mouthwashes[9].

The literature has demonstrated that the incorporation of F released from the restorative material reduced the solubility of dental tissue in acidic environments, this property being based on the F capacity to incorporate itself into the crystalline structure of the hydroxyapatite of the dental hard tissue, resulting in a mineral phase which was less soluble and more resistant to the cariogenic challenge[15].

Accordingly, it was expected that the F-releasing materials evaluated (GTF, GSAF and GV) would demonstrate some anti-cariogenic potential. In the *in vitro* study, after evaluating white spot formation, the presence of caries lesions was found in all the groups, except for GV, which was to be expected in view of its high F release, higher indeed than in groups GTF and GSAF.

The analytical technique chosen for evaluating mineral loss in the enamel blocks *in vitro* was the visual white spot analysis - showing the first clinical evidence of the presence of dental caries. White spot lesions are characterized by the presence of an apparently intact, external surface while the subsurface is seen to be demineralized. This methodology is low-cost and easy to perform, provided that it is carried out by a calibrated examiner, although it is less sensitive than more complex laboratory evaluations such as hardness determination[3] that was used in the *in situ* study; transverse microradiography[8]; polarized light microscopy[16], and quantitative light-induced fluorescence[17]. Although hardness testing was available in our laboratory, hardness was not determined for the *in vitro* study because the specimen was a whole tooth and not an enamel fragment. Thus, the area around the bracket was not flat, making it impossible to evaluate the surface hardness.

Given the results of the *in vitro* study, it was expected that the anti-cariogenic potential of group GV would be corroborated in the *in situ* study, and it was suspected that groups GTF and GSAF would demonstrate some effect on the control of mineral loss, as a more sensitive methodology - namely surface hardness - was used. It was, however, found that there was mineral loss in all groups after the cariogenic challenge *in situ*, with no significant difference between one group and another, demonstrating that the materials evaluated did not have anti-cariogenic potential. Considering that the action of F in controlling caries lesion development is well established, and that previous reports have shown that resin-modified glass ionomer cements[6] and adhesive systems containing F[17] could reduce mineral loss around the brackets, it might be suggested that the protocol used in the *in situ* study was not suitable for evaluating the anti-cariogenic potential of the materials.

The present study, however, was not alone in obtaining results that differed from expectations. In the study of Silva Fidalgo et al.[16], although the specimens were exposed to fluoridated dentifrice and mouthwash, there was mineral loss in all groups, even in those in which F-containing material was used (resin-modified glass ionomer cement and resin cement containing F). The mineral loss was evaluated by means of polarized light microscopy.

This diversity of results led the authors of the present study to questioning the protocols used. These are usually based on studies that evaluated the effect of fluoridated products on the dental surface, or the effect of F released by restorative materials on the adjacent dental area. In other words, they were not designed to evaluate materials for bonding orthodontic brackets.

Many factors may affect the results, such as the amount of material used for bonding, the size of the dental area exposed; distance of indentations in relation to the material; duration of each experimental phase; exposure of volunteers to other sources of F, etc.

With reference to the amount of material used for bonding (which directly affects the quantity of F released) – not only is this a difficult aspect to fully standardize, but it could also vary between operators. Normally, a small quantity is applied to the base of the bracket that is pressed onto the tooth, and any is being removed. One option would be to apply the same quantity to all the brackets (by weighing) and use less pressure on the teeth so that none of the material needs to be removed. This was not done in the present study, nor has it been reported in other studies.

The size of the exposed dental area is standardized by using adhesive tape and nail varnish. Standardization in the preparation of the pieces of adhesive tape is fundamental. One factor that complicates the process is the fact that the bases of the brackets are neither completely flat nor rectangular (to enable the teeth to be better accommodated), and there are variations in size, even when they are designed for the same dental group. Thus, standardizing the type of bracket used in the studies minimizes this variability.

In relation to indentation distances (at 100 µm, 200 µm and 300 µm from the base of the brackets), these differ from the study of Pascotto et al.[6], that used distances of 100 µm and 200 µm only. There is a possibility that at the distance of 300 µm F would have had no influence. In the present study, however, there was no significant difference among the distances, the mean of the three being calculated.

As regards the duration of each experimental phase - five days in the case of the *in situ* study - it could be seen that the peak of F release by the materials occurred in the first 24 hours[8,9]; so that the fragments in the present study were exposed to this peak exposure to F. Five days is in fact a short time when compared with other studies[13], nonetheless it was sufficient to cause mineral loss, which was not controlled by the presence of the F released.

The fact that the volunteers in the *in situ* study did not use fluoridated dentifrice during the experimental phases definitely overestimated the mineral loss that would occur in actual conditions, seeing that the vast majority of toothpastes sold in Brazil contain F. In view of the fact that the materials used to bond brackets succeeded in incorporating and releasing the F to which they were exposed in the oral cavity, even when they did not contain F in their original composition[9,16], future studies should consider using a standardized fluoridated toothpaste or additional experimental groups.

Considering the aspects covered, it is clearly important to develop an effective *in situ* protocol to evaluate the anti-cariogenic potential of materials for bonding brackets, which takes all of these factors into account.

Relative to the bond strength results in the *in vitro* study, a significant difference was found between the materials evaluated. The resin cement containing F (GTF) was similar to the traditional cement (without F; GT), widely used in orthodontic clinics and within the parameters ranging between 6 and 8 MPa advocated in the literature[10].

Whereas, resin cement with its adhesive system that contains F (GSAF) and glass ionomer cement (GV), both produced poor, less than desirable results, although there was no statistically significant difference between groups GTF and GSAF. The absence of any difference between groups GTF and GSAF may have been influenced by the high variability between specimens, reflected by the high standard deviation values. The authors of the present study believed that with a smaller sample size, group GTF might have presented statistically higher results than group GSAF. This is because the material in group GTF had a conventional adhesive system in which an acid was used with a pH lower than that contained in self-etching adhesive systems, such as GSAF, favoring micro-mechanical interlocking, and consequently, the bond strength to tooth enamel[18-20]. Self-etching adhesive agents do not require the separate application of an acid to produce porosity in the substrate. Their formulations incorporate acidic resin monomers that simultaneously demineralize and infiltrate into the dental tissue. Consequently, they should not be, nor do they need to be washed off the surface of the cavity as they cause limited mineralization of the dental tissue. Therefore, these materials are not expected to be as effective in the bonding of brackets onto the enamel[21].

If, at first glance, high bond strength values might seem desirable for cementing brackets, because they would be capable of withstanding the different types of stress induced by the archwires and chewing forces, it should be noted that the greater the force required to remove the bracket the greater the possibility of structural damage to the enamel, which could be difficult to resolve in esthetic areas, and would potentially require repairs to be made[22]. Therefore, the ideal bond strength values for orthodontic practice should fall within the range between 6 and 8 MPa.

Nonetheless, there are reports, such as that by Penido et al.[20], of good performance with self-etching primers. These authors showed acceptable performance of this material when evaluated *in vitro*, with or without thermal cycling after bonding (results of 7.11 and 7.35 MPa, respectively). The different results between the present study and that of Penido et al.[20] may be due to a variety of factors related to the methodology used (substrate, operator, type of bracket), but where these two studies really differed was in the performance of pH cycling, which was not carried out by Penido et al.[20]. The objective of this cycling was to simulate cariogenic conditions with the aim of evaluating the anti-cariogenic potential of the materials. There is no guarantee, however, that the mineral loss occurring in the area of the exposed enamel did not affect bond strength results.

Furthermore, in the study by Montasser, Taha[21], although the use of self-etching primer was accompanied by lower bond strength values than those of the conventional system, the absolute values were always higher than the 6 to 8 MPa limit. It should be remembered that these authors did not carry out any form of cycling (thermal or pH), with the brackets being debonded after 72 hours.

Therefore, the low absolute bond strength values in group GSAF should be considered with caution, because it is too soon to say definitively that resin cement with a self-etching adhesive system is unsuitable for the bonding of orthodontic brackets. The greater practicality of use is a huge advantage and deserves to be studied in more depth.

Lower bond strength values were to be expected for the resin-modified glass ionomer cement, although differing from the present study, there are reports in the literature of its acceptable performance even when the material was exposed to pH cycling[14] or thermal cycling[23]. Although there is no consensus in the literature, it has also been reported that the resin-modified glass ionomer cement could leave less remnant material on the enamel after debonding, but its poor performance was viewed with some reservations[16,23].

The majority of GT fractures occurred within the adhesive between the enamel and the bonding material (41.67%), similar to that found in the study by Wunderlich-Junior et al.[4], though in disagreement with the study of Souza et al.[22], who found 80% of adhesive failures between the bonding material and the bracket using the Transbond XT system. Cohesive enamel fracture was observed in 33.33% of specimens bonded using Transbond XT and 8.33% of the specimens bonded using Transbond SEP, differing from the results found by Campista et al.[24], who detected no cohesive fracture in the enamel for the same materials studied. In the present study, it was only in group GV that no cohesive fracture of the enamel was observed, unlike groups GT, GTF, and GSAF. The study of Arhun et al.[25] suggested that for a cohesive fracture of the enamel to occur, the bond strength of the material must exceed 14.0 MPa, which was observed in groups GT and GTF, where values of up to 25.27 MPa and 14.42 MPa, respectively, were observed; however cohesive fracture of the enamel was observed in group GSAF, although the value of 14 MPa was not exceeded.

With regard to the results of the *in situ* study, after the shear test it was found that all the materials presented bond strength values higher than the 6 to 8 MPa range; these values being sufficient to perform orthodontic treatment, as they will withstand the mechanical traction forces applied to the teeth without breaking the bond.

As regards the type of fracture, the majority of failures in groups GT, GSAF and GV were of the cohesive type in resin, with no significant differences. Adhesive fracture would be ideal due to the fact that there would be no resin remaining on the enamel after the orthodontic brackets are removed; hence it would not be required to remove the cement with finishing burs, which would be advantageous from a clinical point of view. Cohesive fracture of the enamel only occurred in group GSAF (27.3%) and it may be considered that this type of fracture occurred due to the high mean bond strength values (13.2 MPa), considered to be above satisfactory clinical parameters. The high mean bond strength values in group GSAF, in particular, differ quite significantly from those found in the *in vitro* study.

The higher bond strength values found in the *in situ* study may have been influenced by a variety of factors, such as the difference between operators (one researcher performed the bonding in the *in vitro* study while another did so in the *in situ* study), which is a factor that is very difficult to control. Another factor was the time during which the specimens were subjected to the cariogenic challenge; in the *in vitro* study, it was 14 days, while the *in situ* study it lasted 5 days.

Therefore, the establishment of study protocols is important, particularly for *in situ* studies. Nevertheless, when the intention is to evaluate materials for bonding orthodontic brackets, there is a predominant trend towards conducting exclusively laboratory studies (*in vitro*). This makes it necessary to encourage *in situ* studies, place value on them, and propagate the idea that conducting *in situ* studies may not only generate results that would be more consistent with events in clinical practice, but would also be more applicable to this reality.

CONCLUSION

With regard to the F-containing materials evaluated, used for brackets bonding, the resin-modified glass ionomer cement (GV, Vitremer) showed the highest anti-cariogenic potential in the *in vitro* study. However, this was not confirmed by the *in situ* study. Relative to the bond strength values from the *in situ* study, all materials showed that they were adequate for clinical practice.

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CONFLICTS OF INTERESTS

The authors declare no conflicts of interest.

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