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ORIGINAL ARTICLE

Influence of different acid etching times on the shear bond strength of brackets bonded to bovine enamel

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KEYWORDS

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Abstract *Introduction:* The most used product for surface acid conditioning for enamel is 37–40% phosphoric acid, which promotes greater mechanical retention.

Aim: The objective of this study was to compare the shear bond strength (SBS) of brackets bonded to bovine enamel with different acid conditioning protocols and to analyze the surface morphology.

Materials and methods: 169 teeth (n = 13) were divided into 4 groups: control group without conditioning (G1), Dental Gel 37% phosphoric acid (Dentsply) (G2), Ultra Etch 35% (Ultradent) (G3) and Attaque gel 37% (Biodinâmica) (G4). Groups G2, G3 and G4 were subdivided according to the conditioning time into: 10 s (a), 15 s (b), 30 s (c) and 60 s (d). The superficial enamel morphology (n = 3) was analyzed using a scanning electron microscopy (SEM) to analyze the depth of the microporosities. The samples were submitted to the shear test (SBS) with the aid of a universal testing machine (INSTRON) with a speed of 1 mm/min. The enamel after debonding was analyzed to determine the adhesive remnant index (ARI) in a stereoscopic magnifying glass.

Statistical analysis used: The SBS data were analyzed using two-way ANOVA. ARI data were analyzed using generalized linear models and SEM measurements were analyzed using Kruskal

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Wallis and Dunn tests. The 95% significance level was used.

Results: The SBS within G2, G3 and G4 ranged from 11.11 to 12.66 MPa. ARI score 3 was observed in 35% of the samples. The samples analyzed in the SEM showed microporosity depth rangingfrom 1.28 to 2.48 µm.

Conclusions: There was no difference between the acids and times evaluated for SBS. The ARI analysis showed that the studied acids provide protection to the enamel surface, keeping the adhesive attached to the buccal surface after debonding. The increase in conditioning time is directly proportional to the deterioration of the prismatic and interprismatic content.

1. Introduction

Among the different protocols for bonding orthodontic brackets on teeth, the most used product for surface acid conditioning is 37% phosphoric acid, applied for approximately 15 to 30 s produces a high energy hydrophilic surface, thus promotes their mechanical retention (Firoozmand et al., 2013; Tsujimoto, 2016).

This technique of direct bonding of orthodontic accessories offers in comparison to the technique in which bands were used certain advantages such as time-saving, better treatment acceptance, better aesthetics, less risk of decalcifying the enamel, easier control of the bacterial plaque by the patient, lower index gingival irritation, in addition to reduce some of the stages of treatment (Buonocore, 1955; Silva-Benítez et al., 2013; Arash et al., 2017; Hodžić et al., 2018).

However, the type of bonding using 37% phosphoric acid causes enamel mineral loss of around 5 to 50 µm (Legler et al., 1990; Ramesh et al., 2011; Al-Suleiman, et al., 2014), as the amount of mineral loss depends on the concentration of phosphoric acid and the time of application. This procedure received a lot of attention from researchers as the quality of acid conditioning is a crucial factor in the retention of materials (Pithon et al., 2011; Hosseini et al., 2012).

Thus, the error in the acid conditioning protocol is also related to the failure of bonding brackets which occurs in 5 to 7% of cases andleads to a longer clinical time, that cost for the professional and, consequently, for the patient (Deprá et al., 2013). In this sense, it is necessary to constantly improve the techniques and materials used in orthodontics which benefits both patient and professional (Bezerra et al., 2015; Sena et al., 2018).

However, in order to obtain a good result in orthodontic treatment it is important to keep the enamel integrity preserved after the removal of the brackets (Zope et al., 2016). In this sense, the ideal bonding strength for a good orthodontic treatment is approximately 60 Kgf / cm^2 (5.88 MPa) and 80 Kgf/ cm^2 (7.84 MPa) (Reynolds, 1975), yet joints stronger than 14 MPa can cause problems in the enamel surface structure (Sena et al., 2018).

In order to demonstrate the influence of the time of application of phosphoric acid in the bonding of orthodontic brackets, as this is a very important factor within the bonding protocol, the objective of the present study was to compare the shear bond strength (SBS) of brackets bonded in bovineteeth with different acid etching protocols and analyze surface morphology. The null hypothesis is that there is no difference between the different acid conditioning and the evaluated times.

2. Materials and methods

2.1. Sample collection and storage

This study was submitted to the Ethics and Research Institutional Committee and received approval under the number of 095/2017. The sample size was based on a pilot study that determined a sample of 169 bovine incisors (n = 13) with an average age of 24 to 48 months. Inclusion criteria were: healthy teeth free of cavities or cracks and a previous absence of chemical agents (hydrogen peroxide, alcohol or formaldehyde). The teeth were cleaned and they were stored in a 0.1% thymol solution and kept at 4 °C in a refrigerator (Consul, Joinville, SC, Brazil).

2.2. Preparation and division of the specimens

The root portions were sectioned from the coronary portions which were fixed with self-curing acrylic resin Jet (Classic, São Paulo, SP, Brazil) in PVC pipes (25x 10 mm) with the buccal face exposed to the external environment. For this, the vestibular face was in contact with a wax sheet NewWax No. 7(Technew, Rio de Janeiro, RJ, Brazil) The teeth were distributed in 13 groups (n = 13) and allocated in negative control group without acid etching and 12 experimental groups, in which etching time and type of acid varied.

2.3. Preparation of specimens

The buccal surfaces of bovine teeth received prophylaxis with fluoride-free pumice paste with extra fine granulation (S.S. White, Rio de Janeiro, RJ, Brazil) and distilled water using a rubber cup (Microdont, Socorro, SP, Brazil) mounted in contra-angle at low rotation speed (DX, Ribeirão Preto, SP, Brazil) for 10 s. Then, the teeth were washed in running water for 10 s and dried with jets of compressed air (Dental Air, Limeira, SP, Brazil) free of oil for the same time (Scribante et al., 2013a).

Each experimental group was subjected to acid conditioning with a specific acid brand at the different times evaluated. Phosphoric acids were used for acid enamel conditioning: Dental Gel 37% (Dentsply, York, PA, EUA), Ultra Etch 35% (Ultradent, South Jordan, UT, USA) and Attaque Gel 37% (Biodinâmica, Ibiporã, PR, Brazil). After acid conditioning, the surfaces were rinsed in abundance for 10 s and dried for the same time with an air jet. Subsequently, the Transbond XT adhesive system (3 M/ESPE, St. Paul, MN, USA) was

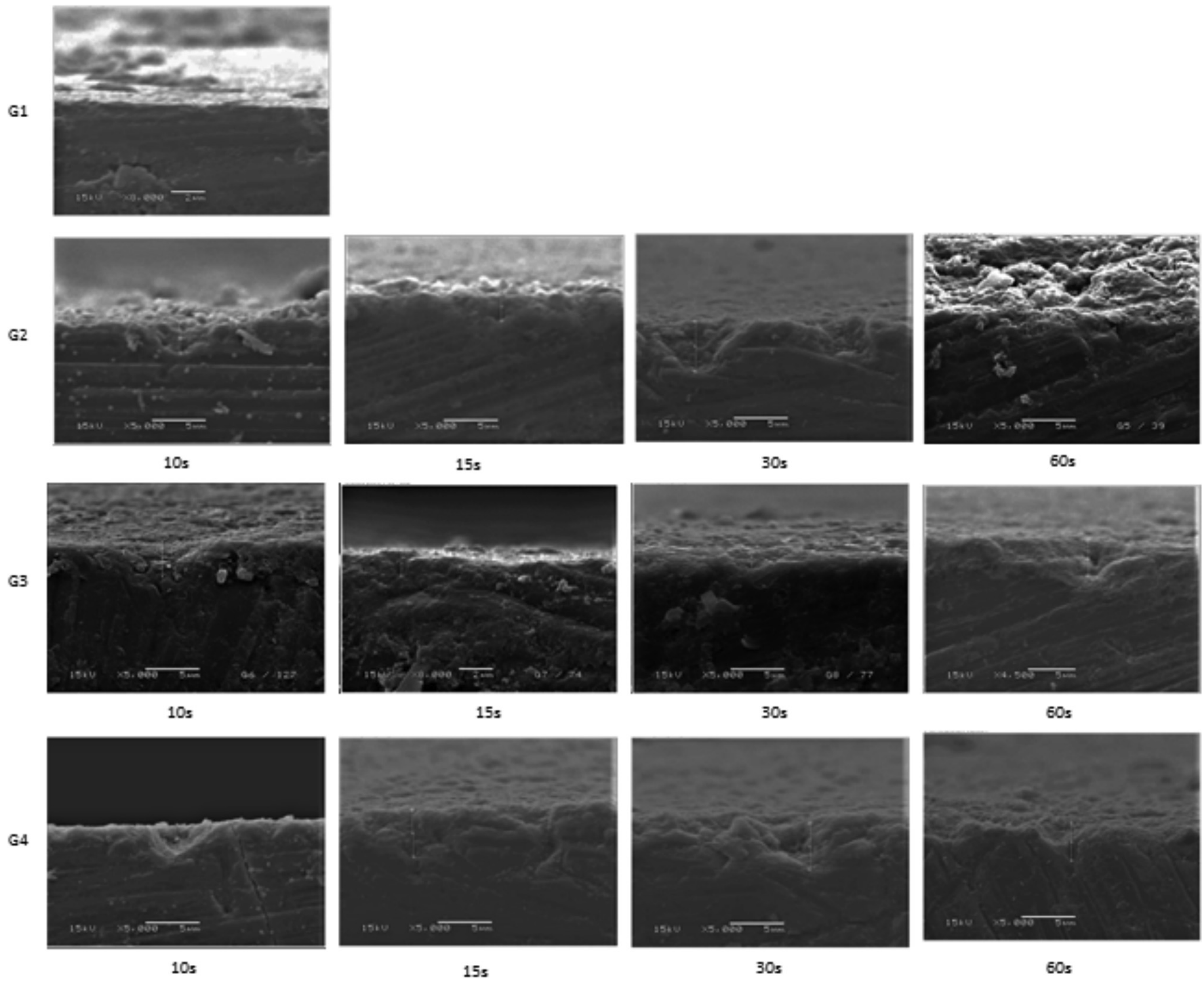

Fig. 1 Microporosities on the bovine enamel surface analyzed by scanning electron microscopy (SEM) after conditioning by 3 different acids (G2, G3 and G4) in 4-time intervals (10 (a), 15 (b), 30 (c), 60 (d) s).

applied to the enamel surface with the aid of a microbrush applicator (KG Sorensen, Cotia, SP, Brazil) and photoactivated by 20 s using the Emitter C device (Schuster, Santa Maria, RS, Brazil) at a power of 832 mW/cm^2. (Giannini & Francisconi; 2008; Correr-Sobrinho et al., 2002).

The Transbond XT orthodontic resin (3 M/ESPE, St. Paul, MN, USA) was applied to the base of the metal brackets Roth Max slot 22 (Morelli, Sorocaba, SP, Brazil). The brackets were bonded in the center of the conditioned area using bracket forceps (Morelli, Sorocaba, SP, Brazil) and pressed to the tooth surface with a weight of 300 g using the Gilmore Needle. The excess of orthodontic resin was removed with the use of dental explorer N^o. 5 (Golgran, São Caetano, SP, Brazil) (Hosseini et al., 2012).

Photoactivation was performed with the Emitter C device (Schuster, Santa Maria, RS, Brazil) for 40 s, with 10 s on each bracket face at a power of 832 mW/cm^2 conferred to each 10 collages with radiometer (Ecel RD7, Ribeirão Preto, SP, Brazil) (Fig. 1). After the bonding of the brackets, the specimens were placed in distilled water for 24 h in an oven at 37 °C in order to promote rehydration and simulate oral conditions (Costa, 2015).

2.4. Shear force analysis

The samples were submitted to the shear bond strength test on the universal testing machine (model 4411; Instron Corp, Norwood, MA, EUA). The active chisel tip was positioned at the tooth/bracket interface and the shear bond strength test performed at a speed of 1 mm/min (Eslamian et al., 2015).

The shear bond strength values were recorded in Kgf and then the shear bond strength value was calculated in Megapascal (MPa) through the formula: $S = L/B$ (S = shear bond strength), L = load required to break the bracket-tooth joint, B = bracket area). The bracket area considered was 12.75 mm^2.

2.5. Adhesive remnant index analysis (ARI)

After the SBS analysysthe surface of the dental enamel was analyzed using a stereoscopic magnifying glass with a 25X magnification in order to determine the amount of adhesive remaining on the enamel surface with the use of the Artun and Bergland, 1984, score:

• Score 0 - absence of any residue from the adhesive layer on the enamel;

• Score 1 - presence of less than half of the remaining resin in the enamel;

• Score 2 - presence of more than half of the remaining resin in the enamel;

• Score3 - presence of all the resin remaining in the enamel withthe impression of the bracket base design.

2.6. Scanning electron microscopy analysis

The slices (5 mm) were dehydrated after fixing and washing them in deionized water for 10 min following the sequence of ethyl alcohol 50%, 70%, 90% for 20 min in each percentage. The conductive coverage of the specimens was made by double-sided conductive carbon tape and covered with gold.

The specimens were examined by SEM (JSM 5600 LV; JEOL, Tokyo, Japan) with 5000x magnification and operated at 15 kV by the same operator. After, Image J software (National Institutes of Health, Maryland, USA) was used to measure the depth of the enamels surface.

2.7. Statistical methodology

A descriptive and exploratory analysis of the data was performed. The SBS data were analyzed using two-way ANOVA. ARI data do not meet the assumptions of ANOVA and were analyzed using generalized linear models and SEM measurements as they do not meet the assumptions and do not fit a known distribution were analyzed using Kruskal Wallis and Dunn tests. The 5% significance level was used.

3. Results

Table 1 shows that there was no statistically significant difference between the acids and the evaluated times regarding shear bond strength ($p > 0.05$). However, all groups differed significantly from the negative control group (without acid conditioning) at all times ($p < 0.05$).

The Table 2 showed that the groups that received Acid 3 had the highest indexes of the adhesive remaining and that the lowest indexes were observed within 15 s. The table 3 shows the median adhesive remaining index for each group.

The Table 4 showed that in the G2 group the microporosity measurements were significantly greater in the time of 30 s (c) than in the time of 10 s (a) ($p < 0.05$). The measurements for G3 were significantly greater at 60 s (d) than at 30 s (c) ($p < 0.05$). For the G4 group, there was no significant difference between the times ($p > 0.05$). In the comparison between the acids, it was observed that in the 10 and 15 s (a and b respectively) there was no significant difference between the three groups ($p > 0.05$). At 30 s (c), microporosity was signif-

Table 1 Average (standard deviation) of shear bond strength in MPa as a function of acid and time.

Time (s)	Acid		
	G2	G3	G4
10	*12.52 (2.27) Aa	*12.61 (1.84) Aa	*12.39 (1.76) Aa
15	*12.55 (2.12) Aa	*12.66 (1.89) Aa	*12.14 (2.55) Aa
30	*12.66 (3.73) Aa	*11.92 (2.82) Aa	*11.11 (2.31) Aa
60	*12.18 (2.07) Aa	*11.40 (1.64) Aa	*12.19 (1.95) Aa

Average (standard deviation) of the G1 control group (without acid conditioning) = 0.97 (0.27). * It differs from the control group ($p \leq 0.05$). Averages followed by the same letters do not differ ($p > 0.05$). p (acid) = 0.5943; p (time) = 0.6109; p (acid $\times$ time) = 0.8880. Uppercase letters represent comparisons between acids at the same time (horizontal). Lowercase letters represent comparisons between times for the same acid (vertical). Note: (G2) – Dental Gel 37% phosphoric acid (Dentsply), (G3) – Ultra Etch 35% (Ultradent) and (G4) – Attaque gel 37% (Biodinâmica).

Table 2 Distribution of the adhesive remaining index as a function of acid and time.

		Score				
Acid (Group)	Time (s)	0	1	2	3	Total
No acid (G1)	–	10	0	0	0	10
Acid 1 (G2)	10 s	1	1	6	2	10
	15 s	4	1	1	4	10
	30 s	3	0	5	2	10
	60 s	0	0	4	6	10
Acid 2 (G3)	10 s	0	5	4	1	10
	15 s	6	0	2	2	10
	30 s	0	1	4	5	10
	60 s	0	3	6	1	10
Acid 3 (G4)	10 s	0	0	4	6	10
	15 s	2	1	1	6	10
	30 s	1	0	4	5	10
	60 s	1	0	3	6	10

Note – Phosphoric acids: (G2) – Dental Gel 37% (Dentsply), (G3) – Ultra Etch 35% (Ultradent) and (G4) – Attaque gel 37% (Biodinâmica).

Table 3 Median (minimum value - maximum value) of the adhesive's remaining index as a function of acid and time.

Time (s)	Acid			
	G2	G3	G4	
10	*2,0 (0,0 – 3,0)	*1,5 (1,0 – 3,0)	*3,0 (2,0 – 3,0)	a
15	*1,5 (0,0 – 3,0)	*0,0 (0,0 – 3,0)	*3,0 (0,0 – 3,0)	b
30	*2,0 (0,0 – 3,0)	*2,5 (1,0 – 3,0)	*2,5 (0,0 – 3,0)	a
60	*3,0 (2,0 – 3,0)	*2,0 (1,0 – 3,0)	*3,0 (0,0 – 3,0)	a
	B	B	A	

Median (minimum - maximum) of the control group (without acid conditioning) = 0.0 (0.0–0.0). * It differs from the control group ($p \leq 0.05$). Medians followed by different letters (uppercase in the horizontal and lowercase in the vertical) differ from each other ($p \leq 0.05$). p (acid) = 0.0093; p (time) = 0.0238; p (acid × time) = 0.1139. (G2) – Dental Gel 37% phosphoric acid (Dentsply), (G3) – Ultra Etch 35% (Ultradent) and (G4) – Attaque gel 37% (Biodinâmica).

Table 4 Median (minimum–maximum value) of the measurements of the enamel microporosity – SEM (μm) as a function of acid and time.

Time (s)	Acid		
	G2	G3	G4
10 s	*1.50 (0.74–3.18) Ab	*1.74 (0.83–3.06) Aab	1.38 (0.74–2.93) Aa
15 s	*1.74 (0.74–3.47) Aab	*1.78 (1.09–2.91) Aab	*1.74 (1.07–4.09) Aa
30 s	*2.40 (1.74–5.99) Aa	1.28 (0.50–3.02) Bb	*1.80 (0.50–3.80) ABa
60 s	*1.84 (0.74–3.02) Bab	*2.48 (1.40–5.12) Aa	*1.96 (1.57–2.77) ABa

Medians (minimum - maximum value) of the control group (without acid conditioning) = 0.0 (0.0–0.0). * It differs from the control group ($p \leq 0.05$). Medians followed by different letters (upper case comparing horizontally between acids and lower case comparing vertically between times) differ from each other ($p \leq 0.05$). (G2) – Dental Gel 37% phosphoric acid (Dentsply), (G3) – Ultra Etch 35% (Ultradent) and (G4) – Attaque gel 37% (Biodinâmica).

icantly higher in G2 than in G3 ($p < 0.05$) andat 60 s it was significantly higher in G3 than in G2 ($p < 0.05$). With the exception of groups G3 at 30 s and G4 at 10 s, the others differed significantly from the control group ($p < 0.05$) (Fig. 1).

4. Discussion

The results of the present study showed no significant difference between acids at different timeswhich led to the acceptance of the null hypothesis. Thus, the values obtained regarding the shear bond strength did not obtain a relevant variation with approximately 12 MPa which is in accordance with the data presented in the literature (Arash et al., 2017; Sena et al., 2018) which present an average resistance to shear of 14.80 MPa. On the other hand, higher values for shear bond strength were also found in the researched literature with values ranging from 13.78 MPa to 34.3 MPa (Naidu et al., 2013; Scribante et al., 2013b; Sfondrini et al., 2013; Najafi et al., 2015; Zope et al., 2016).

This increase in results may be related to differences in the methodology used by the authors of the reference as a way of making and storing the samples. By this analysis, it appears that a shorter conditioning time is sufficient to exert a resistance to peeling without promoting excessive demineralization which results in greater protection to the enamel structure with saving clinical time and reducing the risk of contamination of conditioned áreas (Zope et al., 2016; Arash et al., 2017).

The same way values below that found in this work were also observed with megapascal measurements from 5.62 to 11.90 (Bezerra er al., 2015; Arash et al., 2017; Hodžić et al., 2018). Below expected values can be explained by the due to the lack of preparation of the sample enamel surfaces was done. In this work, the specimes was send the sanding and polishing, for the standardization of the sample, however there was no removal of the superficial enamel, but a more regular surface for bonding the brackets. The protocol used in this study is based on the Alshahrani et al., 2018, where it was observed that only prophylaxis on the superficial enamel promotes provided the highest bracket-enamel bond strength when compared to other protocols.

Although the concentration of Acid 2 is lower than the other acids in this study and in the research cited (Arash et al., 2017; Sena et al., 2018) there was no significant difference in the quality of adhesion of the brackets, because, despite the use of acid in lower concentration, the acid was able to promoted demineralization similar to the other acids studied in the different concentrations evaluated.

Although the temporal comparison of the acids studied in the present study is not available in the literature, in the 30-second interval, Acid 1 showed a greater result in terms of shear bond strength requiring greater mechanical strength to carry out the peeling process in accordance with the literature (Al-Suleiman et al., 2014; Eslamian et al., 2015) in that time interval.

Based on the research by Artun and Bergland, 1984, this characteristic of total permanence of the adhesive adhered to the enamel corresponds to the type 3 score which leads to the aforementioned protection. This aspect was identified by many authors of the reference in line with the results of this research (Yassaei et al., 2014; Zope et al., 2016). The acid that showed the best power to achieve the objective proposed by these authors was Acid 3 with Type 3 score predominance in all evaluated time intervals. In the time interval of 10 s, the type 2 score was predominant in similarity with some evaluated authors (Scribante et al., 2013a; Vinagre et al., 2014; Arash et al., 2017). Regarding the lowest indexes of the adhesive remaining, these were observed in the time of 15 s for the Acid 2 (Lima et al., 2015; Najafi et al., 2015).

Regarding the depth of microporosities, the values found after the analysis of the sample in SEM varied from 1.28 µm, in 30 s of conditioning, to 2.48 µm, in 60 s, both with Acid 3 (Al-Suleiman et al., 2014). The results show that with a shorter conditioning time there is a greater preservation of the integrity of the enamel structure without compromising the efficiency of shear bond strength. Al-Suleiman et al., 2014, found a similar depth to the results obtained in this work using 25% phosphoric acid in the times of 30 s (3 to 4 µm) and 60 s (5 to 10 µm). For the concentration of 37%, the values reached by those authors were not obtainable due to the change in the prismatic and interprismatic content for both the 30-second and 60-second time. Ramesh et al. (2011), using laser confocal microscopy, and Legler et al., 1990, using a calcium chelating solution, showed greater depths: 53.9 µm and 16.7 µm respectively.

As most studies involving acid conditioning are *in vitro* studies, longitudinal clinical studies need to be carried out in order to prove the effectiveness of acid conditioning in less time regardless of concentration in order to complement and validate them.

5. Conclusions

- There was no difference between the acids and times evaluated for SBS.
- The ARI analysis showed that the studied acids provide protection to the enamel surface, keeping the adhesive attached to the buccal surface after debonding
- The reduction in conditioning time is directly proportional to the preservation of the prismatic and interprismatic content without compromising the efficiency of SBS.

Declaration of competing interest

The authors declare there are no potential conflicts of interest with materials involved in the presente investigation.

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