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RESEARCH ARTICLE

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INFLUENCE OF DENTAL SUBSTRATE AND ADHESIVE STRATEGY ON BOND STRENGTH OF TWO UNIVERSAL ADHESIVE SYSTEMS: AN IN VITRO STUDY

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ABSTRACT

The aim of this study is to evaluate the bond strength of two different adhesive systems in bovine teeth, using etch-and-rinse and self-etching technique in enamel and dentin with a bulk fill composite resin. For this purpose, were selected 112 bovine teeth, divided in 8 groups (n=14), being: ECP - enamel conditioned with phosphoric acid; EWA - enamel without prior acid etching; DCP - dentin conditioned with phosphoric acid and DWA - dentin without prior acid etching the groups corresponding to each adhesive tested. The surface treatment was carried out according to the manufacturer's instructions for each group. Three bulk fill composite resin cylinders were fabricated in each tooth, totaling 42 cylinders per group. After 24 hours, the micro-shear bond strength test was performed. The data was analyzed with a Shapiro-Wilk test, followed by analysis of variance and Tukey's post-test. There was a significant difference in the group corresponding to the Single Bond Universal adhesive ECP, obtaining better results. In the intergroup comparison, the two tested products showed no statistical difference between them. The universal adhesives can be used obtained satisfactory results in both techniques. The Single Bond Universal demonstrated a more effective bonding when used acid enamel conditioning on the enamel.

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INTRODUCTION

Dental adhesives are widely used in dental practice, as they offer a bond between dental substrate and composite resin (Pupo *et al.*, 2017). In addition, they have other functions, such as treatment of dentin hypersensitivity, adhesion of intra-root pins, orthodontic brackets, indirect restorations, repair of restorations and aesthetic reanatomizations (Matos *et al.*, 2017). Adhesive systems can be classified into etch-and-rinse and self-etching (Rosa *et al.*, 2015; Kucukyilmaz *et al.*, 2017). The first is characterized by the need of a prior application of phosphoric acid, which completely removes the smear layer and demineralizes the dental substrate. On the other hand in the self-etchers, total acid conditioning is not used and the smear layer is incorporated into the hybrid layer by the infiltration of resin monomers in the collagen meshes (Matos *et al.*, 2017). Currently, there are adhesive systems classified as "universal" or "multimode", allowing the dental surgeon to decide which adhesive strategy to use, etch-and-rinse or self-etching (Kucukyilmaz *et al.*, 2017; Siqueira *et al.*, 2018; Loguercio *et al.*, 2015).

In this system, the bond strength can be achieved by the total conditioning of the acid or not (Matos *et al.*, 2017). Among the components of universal adhesive systems, the 10-MDP monomer stands out, which has the function of an acid binding agent, forming a strong chemical bond with hydroxyapatite of dental surfaces, this substance promotes adhesion by the formation of insoluble salts (MDP- Ca²⁺) (Trevor *et al.*, 2017). A factor that can influence the quality of adhesion of composite resin restorations, in addition to the type of adhesive system, is the contraction of polymerization that can result in gaps in the interface between the restoration and the dental substrate (Benetti *et al.*, 2015; Kasraei *et al.*, 2016). If the contraction force is greater than the adhesion force, it will build up tension to cause micro-cracks or detachment at the adhesive interface (Saeki *et al.*, 2015). To prevent failures in the bonding interface for any commercially available adhesive system, an incremental technique of inserting composite resin (maximum 2mm increments) is the most acceptable because it ensures adequate polymerization without exacerbated contraction. Seeking greater clinical practicality, bulk fill composite resins have recently been developed, which allow insertion

in increments of up to 5mm (Kasraei *et al.*, 2016; Costa *et al.*, 2017). As a result, the objective of this laboratory study was to compare effectiveness of two universal adhesives, using the techniques: etch-and-rinse and self-etch in enamel and dentin associated with bulk fill composite resin. The null hypothesis tested is that the prior acid conditioning step doesn't influence the adhesion of direct restorations in composite resin, when both of the Universal adhesive are used.

MATERIALS AND METHODS

According to the sample calculation, using a completely randomized design with the support of the Bioestat 5.3 program (Instituto Mamirauá – Belém, PA, Brazil) a minimum of 6 teeth per group was defined, with analysis power equivalent to 90% and standard error of 0.01. For greater safety, 112 teeth were selected, divided into 8 groups (n=14) according to the adhesive strategy, the dental substrate and the tested material. 112 bovine anterior teeth were selected, from a local slaughterhouse, freshly extracted and sectioned in high speed rotation with abundant cooling with a diamond bur n° 4138 (KG Sorensen), separating the crowns from the roots. The crowns were fixed in PVC tubes standardized with acrylic resin, leaving the vestibular surface free. Then, the specimens were divided into 8 groups: 4 corresponding to the Universal Single Bond adhesive – SB, and 4 referring to the Ambar Universal adhesive - AM. Being: Being ECP: Enamel conditioned with phosphoric acid; EWA: Enamel without prior acid etching; DCP: dentin conditioned with phosphoric acid and DWA – dentin without prior acid etching (Figure 1).

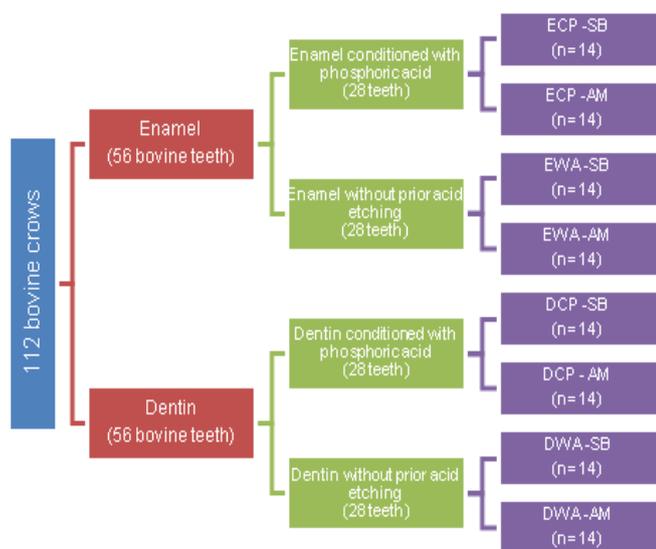


Figure 1. Experimental groups flow diagram

The surfaces of bovine teeth have been worn with the help of a horizontal polishing machine (APL-4, Arotec S.A. São Paulo-SP, Brazil) under water cooling until a smooth enamel and dentin surface is obtained, according to the corresponding group. Posteriorly, the members of the ECP and DCP groups were subjected to acid attack with 37% phosphoric acid, Condac37 (AM, FGM; Joinville, SC, BR), following the manufacturer's instructions. (Table 01). The Ambar universal adhesive (AM, FGM; Joinville, SC, BR) and Universal Single Bond (3M, ESPE, St. Paul, EUA) were applied in the corresponding groups according to the manufacturer's instructions (Table 01).

Then, three cylinders of resin were fabricated in the crowns of each group, totaling 336 specimens. It was used a Tygon matrix (Tygon tubing, TYG-030, Saint-Gobain Performance Plastic, Maime Lakes, FL, USA) with an internal diameter of 2mm and height of 2mm. The matrix was positioned over the surface and its interior was filled with the composite resin Opus Bulk Fill (AM, FGM; Joinville, SC, BR) in a single increment with a spatula (Thompson #6, Miltex, inc., Tuttlingen, Germany). The light activation was performed with the

Bluephase led light cure (Ivoclar Vivadent, Barueri, São Paulo, Brazil) with light intensity of 1200 mW / cm², appliance for 30 seconds. Finally, the matrix was sectioned and removed with a #11 scalpel blade and the specimens were stored at 37°C for 24 hours, being submitted to the microshear bond strength test after this period (Camilotti *et al.*, 2016). The test specimens were coupled to the universal test machine (EMIC DL 500) to measure the microshear bond streng. The test was applied at a speed of 1mm / min with a 50N load cell. The maximum force applied to the base of the cylinders was 45N, 10% less than the load cell value. The data were transformed in Mpa and submitted to statistical analysis. The resin - enamel /dentin fractured interface were analyzed using a stereoscopic magnifier with 100x magnification (Olympus SZ40, Japan).

The types of failure were classified as: Adhesive (A): failure in composite resin-dentin / enamel interface; Mixed (M): failure in the adhesive / enamel-dentin / composite resin interface, which include cohesive failures; Cohesive in composite resin (CC): failure exclusively in composite resin; Cohesive in dentin (CD): fails exclusively within the dentin / enamel (Camilotti *et al.*, 2016). In the statistical analysis, the average values obtained of the bond strength for each specimen of the experimental groups initially underwent the normality curve adherence test using the Shapiro-Wilk test, with a positive result. Subsequently, the selected values were subjected to analysis of variance (ANOVA), followed by the Tukey test (p <0.05) for comparative evaluation.

RESULTS

Anova detected statistically significant differences. By the Tukey test, differences were identified at the level of 5% of significance. The averages with standard deviation are shown in Table 2. In the intergroup comparison there was no statistical difference between the materials and the application techniques of the adhesives in both enamel and dentin. However, comparing the substrates of each material, the only one that showed a difference was the one corresponding to the Single Bond Universal with total acid enamel conditioning (ECP-SB), which presented the highest bond strength, with statistical difference in relation to dentin. After the mechanical test, the fracture pattern was analyzed and classified with the aid of a stereoscopic magnifying glass with a 100x magnification (Olympus SZ40, Japan). There was a predominance of adhesive failures in all groups, as shown in Figure 2.

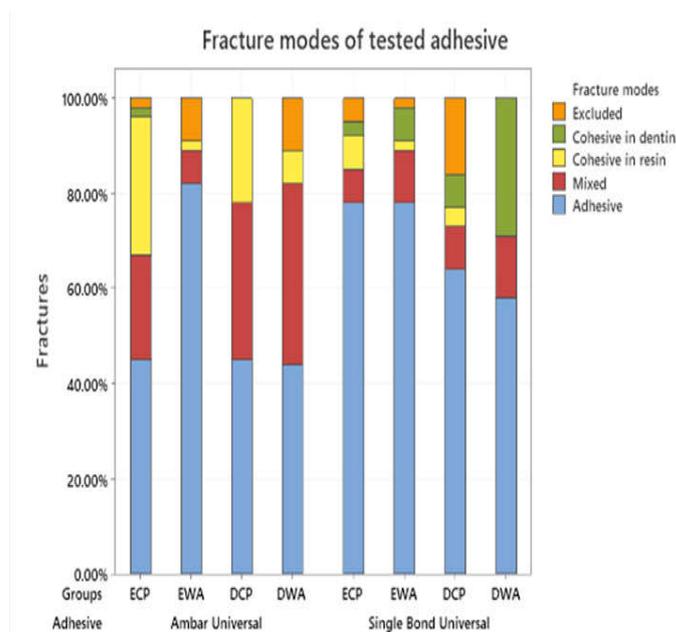


Figure 2. Fracture modes of tested adhesives

Table 1. Instructions for use of the materials used

Material	Instructions for use
Condac37 (AM, FGM; Joinville, SC, BR)	Prepare the area to be etched by cleaning and drying it. The gel should be applied for 15 seconds for both enamel and dentin. After application, the tooth should be rinsed abundantly with water removing completely the acid from the dental surface. The dentin must be dried but not dehydrated.
Single Bond Universal (3M, ESPE, St. Paul, EUA)	Apply the adhesive to the prepared tooth and rub it on for 20 seconds. Gently air dry the adhesive for approximately 5 seconds to evaporate the solvent. Light cure for 10 seconds.
Ambar Universal (AM, FGM; Joinville, SC, BR)	Dispense Ambar Universal in a Dappen pot or directly onto a disposable micro applicator. Apply two layers of adhesive – one drop for each- on the slightly moistened tooth surface. The first layer should be applied vigorously by rubbing the adhesive microapplicator saturated with the product for 10 seconds. The second layer of adhesive – with a new drop – is applied for another 10 seconds and then the area is air blasted gently for 10 seconds to evaporate the solvent. Light cure Ambar Universal with blue light for 20 seconds.

Table 2. Microshear bond strength (Mpa ± DP) – Tukey test (p< 0.05)

	Ambar Universal		Single Bond Universal	
	Conditioned with phosphoric acid	Without prior acid etching	Conditioned with phosphoric acid	Without prior acid etching
Enamel substrate	ECP- AM 30.5700± 8.1368 Aa	EWA- AM 27.6647± 9.1419 Aa	ECP- SB 29.7313± 5.7881 Aa	EWA- SB 21.8087± 10.6140 Aa
Dentin substrate	DCP- AM 28.2614± 9.0935 Aa	DWA- AM 25.6050± 7.7403 Aa	DCP- SB 20.1753± 9.0358 Ab	DWA- SB 22.7193± 6.5683 Aa

*Considering the Tukey test (p<0.05), on each line, the same capital letter do not differ statistically and in each column, the same lowercase letter also do not present a statistically significant difference.

DISCUSSION

The success or failure of a composite resin restoration depends largely on its bond with enamel/ dentin (Shakya *et al.*, 2015). For effective adhesion, the adhesive must completely infiltrate the collagen mesh, sealing and protecting the restoration from possible damage (Matos *et al.*, 2017). The Universal adhesives allow the clinician to choose which adhesive strategy to use, etch-and-rinse with prior application of phosphoric acid or self-etch (Chen *et al.*, 2015). The present study tested two universal adhesives in enamel and dentin, using both adhesive strategies. The tested null hypothesis could not be accepted, once there was a statistical difference between the group corresponding to the Single Bond Universal with total acid conditioning in enamel (ECP- SB), obtaining a higher average bond strength when compared to the other groups (EWA- SB, DCP- SB and DWA- SB). When using the etch-and-rinse technique, the ECP- SB group obtained better results in relation to the self-etch technique. This may have occurred because the enamel has a high mineral content, which allows it to be bonded to the restorative material satisfactorily. (Shakya *et al.*, 2015) Furthermore, conditioning with phosphoric acid may have penetrated to a greater depth resulting in better mechanical retention (Chen *et al.*, 2015; Erickson *et al.*, 2009). Erickson (2009) defend that the self-etch adhesive system is not able to penetrate the enamel as effectively as when using the etch-and-rinse adhesive system. However, Ambar adhesive achieved good results in both adhesive strategies. Some authors consider that the self-etch adhesive system provides a light conditioning property when compared to the technique with phosphoric acid (Kasraei *et al.*, 2016).

Other authors observed a deeper pattern in the enamel layer for various universal adhesives in the conditioning and rinse strategy (Loguercio *et al.*, 2015). Bonding with dentin is a challenge for successful adhesion (Shakya *et al.*, 2015). The results of this study demonstrated intermediate values for dentin, regardless of the adhesive strategy, with the two adhesives. The main obstacle is the heterogeneous nature of this structure, with hydroxyapatite deposited in a collagen fibers and the presence of dentinal tubules. During the adhesion process, minerals are replaced by resin monomers after demineralization, forming a hybrid layer (Shakya *et al.*, 2015). Acid conditioning removes almost all of the hydroxyapatite from the surface, getting only organic collagen what is most challenging for the primary chemical interaction (Hanabusa *et al.*, 2012). The results of a research carried out on bovine teeth obtained greater bond strength in dentin using the etch-and-rinse technique, in comparison

with the self-etch (Camilotti *et al.*, 2016). The work of Cardoso *et al.* (2019), found no significant effect on dentin bond strength of tested universal adhesives, with the exception of Ambar Universal, which had a more stable dentin bond with prior acid conditioning. Another analysis reveals a low-quality bond with dentin when using prior acid etching, although it does not reduce the resistance of immediate adhesion, the author expects the stability of the bond to be less than that produced when a self-etch approach is followed (Hanabusa *et al.*, 2012). Regarding the types of fracture, there was a predominance of adhesive failures in all groups in the present study, in consonance with similar search results (Hanabusa *et al.*, 2012; Ratnaweera *et al.*, 2007). The only adhesive failures demonstrate a real bond strength between the material and the tooth structure (Camilotti *et al.*, 2016) that way, the surface would not be damaged if the restoration failed (Ratnaweera *et al.*, 2007). However, in the study of Cardoso *et al.* (2019) there was a greater number of cohesive dentin failures for the Single Bond Universal adhesive in both adhesive strategies while the Ambar Universal adhesive showed higher frequency of pre-test failures after storage, especially when using the self-etch mode (Cardoso *et al.*, 2019). Although obtained results when applying the Single Bond Universal adhesive, etch-and-rinse technique with acid attack on enamel, have been better when compared to other groups of the same product, there are limitations in tests performed in vitro such as dehydration of the collagen mesh, in addition to the particularities of the oral environment, like temperature, bacteria and natural degradation, and *In vivo* studies are needed to test and predict the behavior of restorations and possible clinical application of this finding.

CONCLUSION

It was found that acid conditioning as a step prior to the use of the Single Bond Universal adhesive shows the best results of bond strength in enamel when compared to dentin. When evaluating the performance of the two adhesives tested, there was no statistically significant difference.

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