The influence of the methodological variables on the shear bond strength

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Abstract

Objectives: Evaluate the influence of several methodological variables on the shear bond strength of *in vitro* studies. **Methods:** 105 bovine incisors were sectioned at the cervical level. The coronary portion was included in PVC rings, fulfilled with auto polymerized acrylic resin, with its labial surfaces positioned upward. All the samples were prepared for bonding, cleaned and acid etched on the central area of the crowns. In this area, central incisors' Morelli™ brackets were bonded with Concise™ (3M/Unitek). Three testing groups were established, according to the studied variable: Group 1—storage previous to bonding (a- thymol 0.1%; b- distilled water; c- freezing); Group 2—crosshead speed of the universal testing machine (a- 0.5 mm/min; b- 1.0 mm/min; c- 2.00 mm/min) and Group 3—commercial brand of 37% phosphoric acid (a- 3M/Unitek; b- Acid Gel; c- Attack Tek). The shear bond strength test was performed at the Emic DL2000™ universal testing machine. Data was analyzed through the Student's t test for independent samples. **Results:** In Group 1, the freezing group showed the higher values of shear bond strength when compared to the others, although no statistical difference was observed ($p > 0.05$). For Group 2, the higher the crosshead speed, the lower the shear bond strength, with no statistical difference. In Group 3, the 3M/Unitek brand showed the highest average of shear bond strength in MPa, but also no statistical difference was shown. **Conclusions:** The variable analyzed in this research had not presented enough influence to determine significant differences between the results.

Keywords: Methodological variables. Investigation protocol. Bracket bonding. Shear bond strength.

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INTRODUCTION

In the last decades, significant changes signed the new directions in the use of orthodontic materials. Among them, might be highlighted the substitution of bands cemented in all teeth by direct bonding in the enamel. Since then, a great variety of materials have been proposed and a significant amount of scientific researches have been done with the intent of improving the bond strength between orthodontic accessories and dental surface, providing a high bond strength during the orthodontic treatment.

In vitro tests represents a reference to discovery, selection and use of dental materials. In Orthodontics the most common test used to determine the bonding systems efficiency is the shear bond strength test. However, the great variability of the results suggests a deficiency in the standardization of the technique, disturbing the correct interpretation of the results and comparisons with another studies as well. Among the variables able to influence the results, there is the storage solution of the teeth before bonding, the time and the type of the surface acid conditioning, the type of the brackets, the crosshead speed of the testing machine and the duration of the storage after bonding.[3,8]

Fox, McCabe e Buckley[8] reviewed 66 manuscripts which evaluated the shear bond strength of orthodontic devices bonded on tooth surface. The authors revealed that there was no consensus about the methodology, showing the necessity of standardization. Therefore, they proposed a protocol to future researches in this area: use surfaces of premolars extracted by orthodontic reasons; use teeth after one month and until six months after extraction, stored in distilled water; after bonding the specimens should be stored at 37ºC during 24 h; the debonding should be performed in Instron machines or similars, with a crosshead speed of 0.1 mm/min; ensure the load of debonding in the same direction; use at least 20 and preferably 30 specimens for each test; to evaluate the adhesive failure in the results; to include in the statistical analysis a prediction related with the clinical performance of the material; to quantify the shear bond strength in Newtons or MegaPascals.

Cal Neto and Miguel[3] reviewed the methodologies used for authors of 127 published manuscripts in two international orthodontic journals (Americam Journal of Orthodontics and Dentofacial Orthopedics e The Angle Orthodontist)—the Table 1 shows the results of the analyses of the manuscripts which used *in vitro* shear bond strength tests.

Storage solution of teeth

Among the storage solutions of bovine and human teeth may we mention: the distilled water, which is the most commonly used; the 0.1% thymol solution—which might both disinfect and conserve the specimens—; 0.5% chloramines solution; physiologic solution; freeze-dry and freeze-wet with immersion on physiologic solution.

Wang and Sheen[19] selected 20 premolars extracted for orthodontic reasons and stored them into water or physiologic solution, to evaluate

TABLE 1 - Variables analyzed by Cal Neto e Miguel.[3]

VARIABLE	PERCENTAGE OF THE MANUSCRIPTS		
substrate for bonding	human teeth (68%)	bovine teeth (19%)	-
storage solution	distilled water (43%)	thymol 0.1% (28%)	not mentioned (15%)
type of bracket	metallic (80%)	ceramic (8%)	plastic (1.5%)
crosshead speed	5.0 mm/min. (36%)	1.0 mm/min. (35%)	0.5 mm/min. (20%)
adhesive remnant index	ARI (40%)	modified ARI (22%)	another method (22%)

the effects over the shear bond strength with the enamel treatment by fluoride previous bonding. In the same way, Chaconas et al[4]—with the intent of evaluate the bonding of the ceramic brackets on the surface of 140 human third molars—stored the teeth into 0.9% physiologic solution.

Others authors as Sargison, McCabe and Millett,[15] to evaluate the union strength of resins on enamel, immerged human premolars into formalin and, thereafter, kept those teeth immersed on distilled water on refrigerator. Lindemuth and Hagge[9], with the same aim, used 50 human premolars with storage in water at ambient temperature. Romano et al[14] used 30 teeth which after extraction were stored on plastic recipient with 0.9% physiologic solution being after stored on refrigerator at 4°C.

Feldens et al[6] aimed to evaluate the shear bond strength of brackets bonded on bovine enamel with Fuji Ortho-LC after blood and saliva contamination and stored teeth in 0.5% thymol solution.

Crosshead speed in testing machine

In the laboratory investigations (*in vitro*) which proposes to evaluate the strength union of orthodontic devices on teeth surfaces the use of universal testing machine or similar is indispensable either for shear or tensile tests.[8] Among the adjustments that the machine has, there is the crosshead speed during the test, giving to the operator a large amount of options. However, although it is not a well-discussed issue, the standardization of the crosshead speed is very important to comparison between different studies and has been verified great variations in the literature in relation to speed selection.[3]

Lindemuth and Hagge[9] evaluated the influence of the crosshead speed over the adhesion strength and failure mode of resins on enamel and dentine. The authors used crosshead speeds of 0.1, 0.5, 1.0, 5.0 and 10.0 mm/min. The results shown that there was no statistical significant difference among groups with bonding on enamel, however, the bond strength had significant variation when bonding was made on dentine, showing the lowest values with 0.1 and 1.0 mm/min and the highest values with 1 mm/min.

Acid etching

The acid etching of the enamel surface was introduced more than half a century ago[2] and until nowadays it is essential to perform an efficient and effective bonding. This procedure causes the dissolution of the inorganic component of the enamel matrix, mainly in the interprismatic region, creating micro porosities on the surface, improving the superficial wetting and the penetration of the resin into the enamel. On this way, a mechanical interlock is created between adhesive and tooth.[13]

A commonly fact when the literature is analyzed is the great variation in acid etching pattern used, either for the acid concentration, time of etching or commercial brand used. In the Table 2 may be observed some of those variations on several studies.

Based on the importance of standardization of the tests and the control of the methodological variables to the reliability of the scientific researches, this study aims to evaluate the influence over the shear bond strength of brackets bonded on bovine enamel according to the following variables:

- storage solution of teeth,
- crosshead speed of the testing machine,
- acid etching.

MATERIAL AND METHODS

Selection and inclusion of the teeth

The sample was composed by 105 permanent bovine incisors, with no fractures or caries, obtained from two slaughterhouses. The teeth were extracted and divided into two groups, containing three subgroups each, and the same

TABLE 2 - Storage method, crosshead speed and acid etching used in different studies, showing a great variety in the methodologies applied.

AUTHORS	STORAGE METHOD	CROSSHEAD SPEED	ACID AND COMMERCIAL BRAND
Fox, McCabe, Buckley[8]	distilled water	0.1 mm/min	-
Wang, Sheen[19]	physiologic solution and water	-	37% phosphoric acid Concise - 3M-Unitek
Chaconas, Caputo, Niu[4]	0.9% physiologic solution	2 mm/min	37% phosphoric acid 3M-Unitek, Ormco and MacroChem
Sargison, McCabe, Millet[15]	formaline and refrigerate distilled water	10 mm/min	37% phosphoric acid unknown brand
Lindemuth, Hagge[9]	water	0.1; 0.5; 1.0; 5.0 e 10.0 mm/min	37% Phosphoric acid - Dentsply
Romano et al[14]	0.9% physiologic solution with dry freezing	0.5 mm/min	-
Feldens et al[6]	0.5% thymol	0.5 mm/min	-
Osterle, Shellhart, Belanger[12]	cloramine – T (refrigerate)	1 mm/min	37% phosphoric acid 3M Unitek
Flores, Sáez, Barceló[7]	distilled water	1 mm/min	37% phosphoric acid 3M Unitek and GC (Fuji Ortho)
Surmont et al[18]	0.01% thymol (refrigerate)	0.5 mm/min	37% phosphoric acid 3M Unitek, Lee, AMC, "A"-Co and Kuraray
Meehan, Foley, Mamandras[10]	distilled water	0.5 mm/min	37% phosphoric acid and 10% poly-acrilic acid - 3M-Unitek and GC
Bishara et al[1]	0.1% thymol	0.5 mm/min	37% phosphoric acid and 10% poly-acrilic acid - 3M-Unitek and GC

control subgroup was part of all groups (Table 3). The teeth were segmented around the cervical third of the roots, and only the crown was used for the study. The bonding area was determined on the center of the buccal surface of the teeth. After the crowns were separated from the roots, retentions on the proximal surfaces were created and then each teeth was positioned with its buccal surface over a glass plate to assure that the largest plain enamel surface for bonding was parallel to the ground, and in this position the crown was stabilized with wax and then mounted in self-cured acrylic resin in plastic cylinders of 20 mm X 20 mm. A bubble level was used to assure that the lateral walls of the plastic cylinder were perpendicular to the buccal surface of the teeth (Fig 1), allowing a standardization on the positioning of the metallic matrix during the test.

The teeth from Group 1 were stored in three different storage solution before bonding, in order to evaluate the effects of the storage on the shear bond strength. The teeth from Group 2 were stored in a 0.1% thymol solution before bonding, and for the shear bond strength test three different velocities of the cell charge were used. The teeth from group 3 were also stored in 0.1% thymol solution and three different commercial brands of 37% phosphoric acid were used for acid etching (Table 3).

Sample preparation

The specimens were cleaned with Vaporetto™ to remove the excess of wax from the inclusion

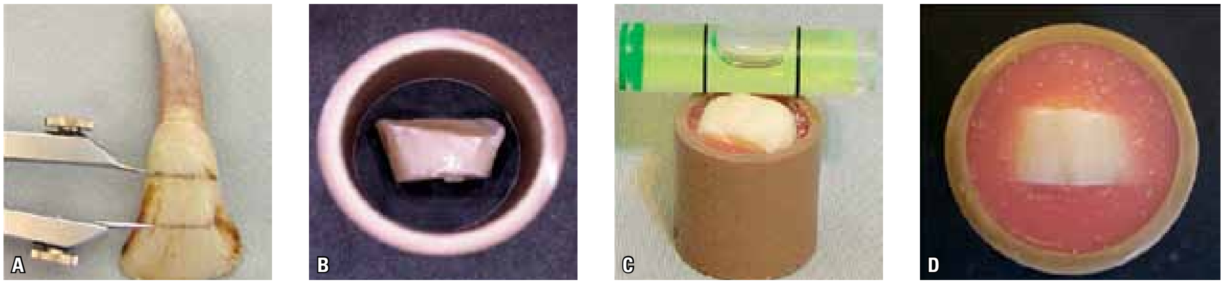

FIGURE 1 - Delimitation of the crown to segmentation (**A**); Plastic cylinder and crown positioned over the glass plate (**B**); Analysis of the parallelism between ground, crown and plastic cylinder (**C**); Specimen ready for bracket bonding (**D**). Source: Sória et al.[17]

process. After the specimens were washed, they were prepared for the bonding procedures as follows:

- Prophylaxis: with rubber cup (changed every each 5 specimens), in a low rotation, using pumice paste during 10 seconds.
- Cleaning: with distilled water during 10 seconds.
- Drying: with air, during 20 seconds, at a 50 mm distance.
- Acid etching: with 37% phosphoric acid during 30 seconds.
- Cleaning: with distilled water during 10 seconds.
- Drying: with air, during 20 seconds, at a 50 mm distance.

Bracket bonding

For the bonding procedure, 105 central incisor brackets (Morelli™) were used and, after the preparation of the sample, the bonding was done as follow (Fig 2):

- Manipulation of the bonding material (Concise - 3M/Unitek, Sumaré, SP, Brazil), according to the manufacturer's instructions.
- Displaying of the bonding material on the bracket base.
- Positioning of the bracket with a bonding nipper.
- Removal of the adhesive excess with a scaler.

TABLE 3 - Distribution of groups and subgroups.
* All of these subgroups are the same, therefore, it is present in all of the groups and was considered the control subgroup.

TOTAL OF TEETH	GROUPS (N)	(N) - SUBGROUPS
105 bovine teeth	storage method (45 teeth)	(15) - thymol 0.1%*
		(15) - distilled water
		(15) - freezing
	crosshead speed (45 teeth)	(15) - 0.5 mm/min
		(15) - 1.0 mm/min*
		(15) - 2.0 mm/min
	commercial brands of acids (45 teeth)	(15) - 3M/Unitek*
		(15) - Acid Gel
		(15) - Attack Tek

- Awaited the time for the autopolymerization of the bonding material, according to the manufacturer.

Storage after bonding

After bracket bonding, the specimens were stored in a closed recipient with 100% relative humidity at 23°C, during one hour, and then the specimens were immersed in distilled water for 24 hours at 37°C.

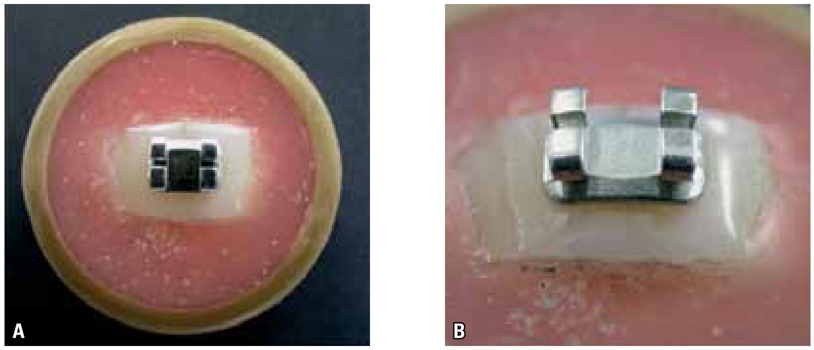

FIGURE 2 - Features of the brackets bonded on the central region of the crown. Superior view (**A**) and approximated inferior view (**B**). Source: Archives of PUCRS.

Mechanical essay – Shear bond strength

A chisel-edge plunger was mounted in the movable crosshead, with a 2 mm thick contact area with the bracket. The matrix was positioned on the universal testing machine (Emic DL2000, São José dos Pinhais, PR, Brazil), connected to a computer with the Mtest software, which registers the maximum load at failure in MegaPascal (MPa).

The control subgroup determined in this investigation was: storage in 0.1% thymol solution, acid etching with 37% phosphoric acid from 3M /Unitek and cell charge of 1.0 mm/min, which are more commonly used in the literature nowadays.

Statistics

Data was statistically analyzed using SPSS™ - Statistical Package for Social Sciences 10.0. To compare the groups, student's t test for independent samples was used. Statistical significance was considered for values of $p < 0.05$ (95% confidence interval).

RESULTS

The results obtained through the Student t test showed that there was no statistical significant difference between the means of experimental groups and between experimental groups and control group, in all of variables analyzed (Tables 4, 5 and 6).

DISCUSSION

An ideal sample to be used in shear bond strength tests on enamel must be composed by human central incisors[12]. However, due to the difficulty to obtain those teeth, a great number of researchers began to use premolars extracted due to orthodontic reasons. Nevertheless, premolars have a large variation in curvature of the buccal surface which adds one more variable in bracket/enamel interface[12]. Some authors argue that the bovine incisors are easily obtained and have a large flat area at the buccal surface; moreover, there is micro structural similarity between human and bovine enamel and, therefore, the bovine teeth have been recommended as a substitute to human tooth in bonding researches.[3,6,11,14]

Laboratory studies are normally performed with the intent of evaluating new techniques or materials, allowing the clinical use thereafter. Beyond the analysis of the results of the studies, there is also the possibility of comparing the results with others studies already performed. However, the absence of well defined protocols to *in vitro* studies allow the application of different methodologies disturbing the comparison between researches and reducing the scientific value of some studies, preventing the results to be extrapolated to orthodontic clinic.[3,5,8]

The storage process of teeth and specimens previously to orthodontic device's bonding could eventually change the characteristics of the enamel surface and, furthermore, could lead to different results in shear tests.[3,5,8] However, the results of the Group 1 (Table 4) shown that the storage solution of teeth did not significantly influenced the union strength. Those results allow to the investigators to choose the storage solution, without great concern about the difference in results.

Due to the viscoelastic pattern of the bonding materials either the union strength or the adhesive failure mode in the debonding can be changed with the crosshead speed variation.[3,8] With lower velocity, for instance, the adhesive might show great deformations, the load might be dissipated and, consequently, the adhesive could support greater load. On this basis, there is a tendency to obtain low values of enamel bond strength when is adopted a high crosshead speed.[9] However, the results of the Group 2 (Table 5) demonstrated that there was no statistically significant differences among the subgroups with different load cell speeds. It might be observed that there was a tendency of reduction in bond strength with the increment in the speed of the testing machine, which suggest that if high values of speed were applied—as 5 or 10 mm/min—possibly would result in mean values with statistical difference between them.

The acid etching with 37% phosphoric acid is essential previously to orthodontic devices' bonding with resin, to obtain more microporosities and mechanical retention between adhesive and tooth surface.[2,13] Nowadays, there are commercial brands of acids with different compositions which can lead to different etching pattern of enamel. In this study were analyzed the brands 3M/Unitek, AcidGel and Attack Tek. The subgroup 3M/Unitek showed the high shear bond strength among the three subgroups, possibly showing a greater surface conditioning of the surface, however, without statistical difference in relation of others subgroups (Table 6).

The variables studied in this study obviously are not alone during the *in vitro* tests, therefore, this data does not discard the possibility of the

TABLE 4 - Means, standard deviations, and Student t test for comparison of experimental subgroups between them and with the control subgroup, in the Group 1 (storage solution).

GROUP	N	MEAN (MPA)	STANDARD-DEVIATION	P
control subgroup (thymol)	15	11.50	6.07	0.85
distilled water	15	11.13	4.70	
control subgroup (thymol)	15	11.50	6.07	0.28
freezing	15	13.51	3.75	
distilled water	15	11.13	4.70	0.14
freezing	15	13.51	3.75	

TABLE 5 - Means, standard deviations, and Student t test for comparison of experimental subgroups between them and with control subgroup, in the Group 1 (crosshead speed).

GROUP	N	MEAN (MPA)	STANDARD-DEVIATION	P
control subgroup (1 mm/min)	15	11.50	6.07	0.69
2 mm/min	15	10.69	4.94	
control subgroup (1 mm/min)	15	11.50	6.07	0.68
0.5 mm/min	15	12.31	4.39	
2 mm/min	15	10.69	4.94	0.35
0.5 mm/min	15	12.31	4.39	

TABLE 6 - Means, standard deviations, and Student t test for comparison of experimental subgroups between them and with control subgroup, in the Group 1 (acids).

GROUP	N	MEAN (MPA)	STANDARD-DEVIATION	P
control subgroup (3M/Unitek)	15	11.50	6.07	0.36
attack Tek	15	9.64	4.77	
control subgroup (3M/Unitek)	15	11.50	6.07	0.43
acid gel	15	9.91	4.63	
Attack Tek	15	9.64	4.77	0.87
acid gel	15	9.91	4.63	

inappropriate standardization influence over the final results of researches, so will be necessary another analyses to complete this understanding. Moreover, it is important to highlight that in this study the variables were analyzed independently, inside each group. However, if the groups were compared among themselves involving more than one variable, could be found different results even with statistical significant difference.

CONCLUSION

Based on the results the following conclusions can be draw:

- The storage solution of teeth previously to inclusion and bonding did not influence significantly the shear bond strength.

- There was a tendency of reduction of the shear bond strength with the increase in the crosshead speed, however, without statistical difference among the groups.

- The different commercial brands of acids also did not show statistical significance influence over the shear bond strength.

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REFERENCES

1. Bishara SE, Olsen ME, Damon P, Jakobsen JR. Evaluation of a new light-cured orthodontic bonding adhesive. Am J Orthod Dentofacial Orthop. 1998 Jul;114(1):80-7.
2. Buonocore MG. A simple method of increasing the adesion of acrylic filling materials to enamel surfaces. J Dent Res. 1955 Dec;34(6):849-53.
3. Cal Neto JOAP, Miguel JAM. Uma análise dos testes in vitro de força de adesão em Ortodontia. Rev Dental Press Ortod Ortop Facial. 2004 jul/ago;9(4):44-51.
4. Chaconas SJ, Caputo AA, Niu GS. Bond strength of ceramic brackets with various bonding systems. Angle Orthod. 1991 Spring;61(1):35-42.
5. Eliades T, Brantley WA. The inappropriateness of conventional orthodontic bond strength assessment protocols. Eur J Orthod. 2000 Feb;22(1):13-23.
6. Feldens JÁ, Freitas MPM, Lima SEM, Oshima HMS. Resistência ao cisalhamento de "brackets" colados em esmalte bovino contaminado por sangue ou saliva. Rev Odonto Ciênc. 2004 abr/maio;19(44):192-96.
7. Flores AR, Sáez EG, Barceló F. Metallic bracket to enamel bonding of photopolymerizable resin-reinforced glass ionomer. Am J Orthod Dentofacial Orthop. 1999 Nov;116(5):514-7.
8. Fox NA, McCabe JF, Buckley JG. A critique of bond strength testing in Orthodontics. Br J Orthod. 1994 Feb;21(1):33-43.
9. Lindemuth JS, Hagge MS. Effect of universal testing machine crosshead speed on the shear bond strength and bonding failure mode of composite resin to enamel and dentin. Mil Med. 2000 Oct;165(10):742-6.
10. Meehan MP, Foley TF, Mamandras AH. A Comparison of the shear bond strengths of two glass ionomer cements. Am J Orthod Dentofacial Orthop. 1999 Feb;115(2):125-32.
11. Nakamichi I, Iwaku M, Fusayama T. Bovine teeth as possible substitute in the adhesion test. J Dent Res. 1983 Oct;62(10):1076-81.
12. Oesterle LJ, Shellhart WC, Belanger GK. The use of bovine enamel in bonding studies. Am J Orthod Dentofacial Orthop. 1998 Nov;114(5):514-9.
13. Owens SE Jr, Miller BH. A comparison of shear bond strengths of three visible light-cured orthodontic adhesives. Angle Orthod. 2000 Oct;70(5):352-6.
14. Romano FL, Tavares SW, Ramalli EL, Magnani MBBA, Nouer DF. Análise in vitro da resistência ao cisalhamento de bráquetes metálicos colados em incisivos bovinos e humanos. Rev Dental Press Ortod Ortop Facial. 2004 nov/dez;9(6):63-9.
15. Sargison AE, McCabe JF, Millett DT. A laboratory investigation to compare enamel preparation by sandblasting or acid etching prior to bracket bonding. Br J Orthod. 1999 Jun;26(2):141-6.
16. Silverman E, Cohen M. Etching versus nonetching. Am J Orthod Dentofacial Orthop. 1998 Jul;114(1):80-7.
17. Sória ML, Menezes LM, Oshima HMS, Rizzatto SMD. Resistência de união de braquetes ao esmalte bovino: avaliação de três cimentos de ionômero de vidro. Rev Dental Press Ortod Ortop Facial. 2003 nov/dez;8(6):89-98.
18. Surmont P, Dermaut L, Martens L, Moors M. Comparison in shear bond strength of orthodontic brackets between five bonding systems related to different etching times: an in vitro study. Am J Orthod Dentofacial Orthop. 1992 May;101(5):414-9.
19. Wang WN, Sheen DH. The effect of preatreatment with fluoride on the tensile strength of orthodontic bonding. Angle Orthod. 1991 Spring;61(1):31-4.

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