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Original Article

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Effect of time and dental substrate on the bond strength of a universal adhesive system: in vitro study

Efeito do tempo e do substrato dental na resistência de união de um sistema adesivo universal: estudo in vitro

Poliana Maria de Faveri CARDOSO[a]*, **Rafael da Silva VANOLLI[a]**, **Fernanda Rafaela RIBEIRO[a]**, **Marcio José MENDONÇA[a]**, **Veridiana CAMILOTTI[a]**

[a]Unioeste – Universidade Estadual do Oeste do Paraná, Centro de Ciências Biológicas e da Saúde, Curso de Odontologia, Cascavel, PR, Brasil

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Resumo

Introdução: Os adesivos dentinários proporcionam união entre o substrato dental e resina composta, porém esta união pode ser influenciada pelo agente de limpeza. **Objetivo:** Avaliar a influência na resistência de união (RU) de um sistema adesivo universal associado à da água ozonizada como solução de limpeza cavitária. **Material e método:** Foram selecionadas 40 coroas de dentes anteriores bovinos divididos em 4 grupos: AD (limpeza dentinária com água destilada) e AO (limpeza dentinária com água destilada ozonizada). Cada grupo foi subdividido em outros dois grupos de acordo com o tempo de armazenamento (24h e 30 dias) armazenados a 37ºC. A limpeza dentinária foi realizada por meio de uma seringa de 60mL de cada solução e fricção por 10 segundos. O sistema adesivo universal selecionado foi aplicado de acordo com as instruções do fabricante e fotoativado por 20 segundos. Em seguida, foram confeccionados 3 cilindros (matriz Tygon com um diâmetro interno de 2 mm e 2 mm de altura) de resina composta nas coroas nas regiões cervical, média e incisal e fotoativados por 30 segundos. Após o armazenamento foi realizado o teste de RU por microcisalhamento e análise do tipo de fratura. Os dados coletados foram submetidos a análise estatística através do teste de Shapiro Wilk com nível de significância de 95%, ANOVA e Tukey. **Resultado:** Houve diferença estatística significativa entre os valores de RU apenas na análise intergrupos para o tempo de 24 horas, entre o terço médio dos grupos (LDAO24 = 2,70 (± 2,39); LDAO30 = 3,82 (± 2,31)). O tipo de fratura predominante para ambos os grupos em todos os tempos foi de fratura adesiva, com exceção ao terço médio e incisal do AD, que foi fratura coesiva de dentina. **Conclusão:** A utilização do ozônio não alterou a resistência de união adesiva.

Descriptors: Resistência de união; adesivos universais; ozônio.

Abstract

Introduction: Dentin adhesives provide union between the dental substrate and composite resin, but this union can be influenced by the cleaning agent. **Objective:** Evaluate the use of ozonated water as a cavity cleaning solution. **Material and method:** 40 bovine dental crowns were selected, divided into four groups: AD (dentin cleaning with distilled water) and AO (dentin cleaning with ozonized distilled water). Each group was divided into two storage periods (24h and 30 days) kept at 37 °C. The selected universal system adhesive was employed according to the manufacturer's instructions and light cured for 20 seconds. At that time, three cylinders were made (Tygon matrix with an internal diameter of 2 mm and a height of 2 mm) of composite resin in the crowns in the cervical, médium, and incisal regions and light cured for 30 seconds. After storage, the bonding strength was tested by micro-shear, and fracture type analysis was performed. The data were submitted to statistical analysis using the Shapiro-Wilk test with a significance level of 95%, ANOVA, and Turkey. **Result:** There was a statistically significant difference between the bond strength values, only in the intergroup analysis for the time of 24 hours, between the middle third of the groups (LDAO24 = 2.70 (± 2.39); LDAO30 = 3.82 (± 2.31)). The predominant fracture type for both groups was an adhesive fracture, except in the medium and incisal thirds of the AD, which was a cohesive dentin fracture. **Conclusion:** The utilization of ozone did not change the bond strength adhesive.

Descriptors: Bond strength; universal adhesive; ozone.

INTRODUCTION

Nowadays, there is a new concept in dentistry called Minimally Invasive Dentistry, which consists of selective removal of carious tissue to preserve tooth structure and prevent accidental exposure of pulp tissue. However, for this technique to be executed safely, it is necessary to use a material that completely seals the cavity and eliminates the microorganisms present in the recently prepared dental element. Meantime, as no materials guarantee this situation in the long term, it is recommended to use cavitary cleaning solutions as disinfecting agents[1,2].

Disinfectant solutions aim to clean the cavity and optimize the action of the bonding agent[3]. In this context, ozone therapy has become widely used in Dentistry due to its biological properties, compatibility, and antibacterial effects[4,5]. The ozonized water works by eliminating the microorganisms due to its highly oxidative action, which causes the dissociation of the bacterial cell wall without promoting bacterial resistance to drugs or substances[6,7].

Beyond the proper cavity cleaning, polymerization shrinkage is another factor that can influence the quality of adhesion of composite resin restorations. The polymerization shrinkage comes from converting the monomers present in the resins into polymers, which can result in gaps at the interface between the restoration and the dental substrate[8,9]. What occurs if the shrinkage stress lives more significant than the bond strength of the adhesive to the substrate, and there is a build-up of tension that can lead to microcracks or detachment at the adhesive interface[10].

To prevent bonding interface failures for whatever adhesive system is available on the market, the incremental technique for inserting the composite resin (maximum 2 mm increments) is the most accepted as it guarantees adequate polymerization without exacerbated shrinkage. In search of greater clinical practicality, bulk fill composite resins were recently developed, which allow insertion in increments of up to 5 mm[8,11].

The bulk fill type of composite resin diverges from conventional ones due to two principal characteristics, its contraction coefficient and translucency. The shrinkage that occurs in bulk fill resins is observed only on the occlusal surface of the restoration. In contrast, in conventional resins, polymerization shrinkage is observed over the entire length of the composite resin. Regarding translucency, bulk fill resins have more translucency, which allows more light passage, making it possible to use increments with greater depth[12].

Therefore, the hypothesis tested in this study is that the dentinal substrate subjected to cavitary cleaning with distilled and ozonated water will not interfere with the bond strength of a universal adhesive associated with a bulk fill resin.

MATERIAL AND METHOD

Experimental Design

The sample calculation was based on probability distributions of family F, with a repeated family delineation, with interaction within and between factors. The effect size used was 0.15, type 1 error (α) of 0.05, and analysis power of 0.85 guaranteeing a minimum of 108 sample units (specimen), with 9 samples per experimental group, for convenience it was decided to make 10 samples per experimental group, totaling 120 specimens. The sample calculation was performed using the GPower program (version 3.1.9.2- University of Düsseldorf, Düsseldorf – Germany).

Study Design

The 40 dental bovine crowns were divided into 2 groups: AD: distilled water solution; AO: ozonized distilled water solution. Each group was subdivided into two other groups according to the storage period (24h and 30 days), onde being kept at 37°C.

Preparation of the Test Samples

Bovine anterior teeth were selected from a local slaughterhouse, freshly extracted, and sectioned in high rotation with abundant refrigeration with a diamond tip, number 4138 (KG Sorensen), separating the crowns from the roots. The crowns were fixed in standardized PVC tubes with acrylic resin, leaving the buccal surface free. The bovine tooth surfaces were ground to a smooth dentin surface.

Posteriorly, the crowns were submitted to dentin cleaning with the aid of a 60 mL syringe and friction with a kg brush applicator (KG SORENSEN; Cotia, SP, BR) for 10 seconds for each solution.

Preparation of Ozonized Water

500 mL of distilled water plus 16 ppm of ozone gas was used with an ozone generator device (Ozone & Life/O&L1.5.0RM, São José dos Campos, SP, Brazil), for 5 minutes, 30 minutes before use.

Adhesive Procedure

Universal Ambar adhesive (AM, FGM; Joinville, SC, BR) was applied to the dentin surfaces of all crowns after the dentin cleaning procedure according to the manufacturer.

Subsequently, 3 composite resin cylinders were made on the crowns of each group. A Tygon (Tygon tubing, TYG-030, Saint-Gobain Performance Plastic, Maime Lakes, FL, USA) matrix was used with an internal diameter of 2 mm and a height of 2 mm. The matrix was positioned with the aid of a clinical tweezer on the surface and its interior was filled with Opus Bulk Fill Flow (AM, FGM; Joinville, SC, BR) composite resin. Photoactivation was performed using a Valo light apparatus (Ultradent Products, South Jordan, UT, USA) with 1400 mW/cm^2 for 30 seconds. Finally, the matrix was sectioned and removed with a scalpel blade number 11 and stored according to each period (24h and 30 days) both at 37°C.

Microhardness Tests

The crowns of each group were subjected to the microshear test in a universal testing machine (EMIC) at a speed of 1mm/min with a load cell of 50N. The maximum force applied to the base of cylinders was 45N, 10% less than the load cell value. Data were transformed into Mpa and subjected to statistical analysis.

Fracture Analysis

The resin-enamel/ fractured dentin interface was analyzed in a stereoscopic magnifier at 100x magnification (Olympus SZ40, 40, Japan). Failure types were classified as:

- Adhesive (A): failure at the composite resin-dentin/enamel interface;
- Mixed (M): adhesive/enamel-dentin/composite resin interface failures, which include cohesive failures;
- Composite resin cohesive (CC): failure exclusively in composite resin;
- Cohesive in dentin (CD): failure exclusively within dentin/enamel.

Statistical Analysis of Microhardness and Type of Fracture

A statistical analysis of the data was performed using Bioestat® 5.3 (Institute for Sustainable Development *Mamirauá,* Tefé, Amazonas, Brazil). Initially, the data were evaluated for the requirement of normal distribution using the *Shapiro Wilk* test, with a positive result. From the analysis of this prerequisite, satistical tests were performed to evaluate the existence of statistically significant differences between the groups through the Analysis of Variance test (ANOVA), followed by the Turkey post-test, p <0.05.

RESULT

The results of the statistical analysis of bond strength for each experimental group are listed in Tables 1, 2, 3 and 4.

The analyses of Table 1 reveal the absence of statistically significant values for the AD group, according to the storage time of the samples, according to the Kruskall Wallis Analysis of Variance test (p <0.05).

Table 1. Median values and interquartile deviation of microshear bond strength (Mpa), for Group 1 according to time

Groups	LDAD24	LDAD30
Cervical	4.46 (± 2.71)	5.89 (± 2.78)
Medium	4.61 (± 2.71)	7.19 (± 1.75)
Incisal	5.25 (± 5.08)	4.77 (± 2.94)

The analysis of Table 2 reveals the absence of statistically significant values for the AO group, according to the storage time of the samples, according to the Kruskall Wallis Analysis of Variance test (p<0.05).

Table 2. Median values and interquartile deviation of microshear bond strength (Mpa) for Group 2 according to time

Groups	LDAO24	LDAO30
Cervical	4.14 (± 1.43)	3.34 (± 2.87)
Medium	2.70 (± 2.39)*	3.82 (± 2.31)*
Incisal	2.86 (± 3.57)	3.82 (± 5.25)

*Means statistically significant difference

Table 3 shows a statistically significant difference in bond strength values in the intergroup analysis for 24 hours, between the middle third of the AD and AO groups, according to the Kruskall Wallis Analysis of Variance test (p<0.05).

Table 3. Median values and interquartile deviation of the microshear bond strength (Mpa), analyzes at 24 hours, according to the experimental group

Groups	Group 1	Group 2
Cervical	4.90 (± 2.00)	3.88 (± 1.57)
Medium	4.84 (± 1.76)	2.99 (± 1.92)
Incisal	5.51 (± 3.05)	3.02 (± 2.31)

Table 4 shows the absence of statistically significant bond strength values in the intergroup analysis for the time of 30 days, according to the Kruskall Wallis Analysis of Variance test (p<0.05).

Table 4. Median values and interquartile deviation of the michoshear bond strength (Mpa), analyzed at 30 days, according to the experimental group

Groups	Group 1	Group 2
Cervical	5.60 (± 2.84)	3.66 (± 2.31)
Medium	7.29 (± 1.39)	4.14 (± 1.78)
Incisal	5.70 (± 2.61)	4.36 (± 2.85)

Regarding the analysis of the fracture in the AD, it was possible to observe a predominance of adhesive fracture for both times in the cervical third and of cohesive dentin fracture for the middle and incisal thirds.

In turn, fracture analysis for the AO group showed a predominance of adhesive fracture for both times in all thirds.

DISCUSSION

The null hypothesis tested in this study was accepted since the ozonated water as a cleaning solution did not interfere with the akturk results, it corroborated the studies by Akturk et al.[13] and Borba et al.[14]. This finding is quite attractive since the more significant amount of O2 in the ozonated water could interfere with the polymerization process of the composite resin and affect the bond strength values[15]. Perhaps 24h storage is enough to eliminate free O2, promoting the promising results found. This way, the antimicrobial advantages of ozonated water can be combined without interfering with the bond strength of the adhesive system and composite resin.

Considering the resistance values achieved when evaluating samples submitted to dentin cleaning with distilled water stores and aged for 24 hours and 30 days are lower than those found in the literature, this fact may have occurred due to the smaller internal diameter and height of the Tygon matrix. This reduction may have influenced the force exerted on the load cell, resulting in the lowest values found[16,17].

The analysis between storage times (24h and 30 days) also did not reveal significant differences. Similar results were found by Dellazzana et al.[18] and Zhou et al.[19] in which time does not influence the fracture resistance of the dentin-restoration interface. Longer storage time could cause differences, as found by Zhang et al.[20].

In the intergroup analysis (Tables 3 and 4), it was possible to observe a significant difference in bond strength only for the 24-hour time between the middle third of the groups, as reported by Rodrigues et al.[21]. This fact can be attributed to the morphological characteristics of dentin, as it presents regional variations in dentinal tubules, moisture, and depth, as well as pathological and physiological changes to which this substrate is subject, making dentin bonding complex and difficult to control[22]. Other studies, using different adhesive systems and composite resins, evaluated the effects of ozone application on the dentin of bovine teeth in terms of shear strength, and no reduction in adhesion was observed between the dental substrate and the resin composite[14,22].

It was observed that the predominant type of fracture in the group in which there was no manipulation of distilled water was cohesive for the middle and incisal thirds, in line with previous studies[21]. In the case of the group in which ozonated water was used, the adhesive fracture was more frequent for all thirds[14,23,].

Further laboratory tests and clinical trials are necessary to confirm the data obtained in this in vitro study. Since micro shear tests examine areas of more excellent bonding and are associated with non-uniform distribution of stresses at the adhesive interface, it is indicated to minimize the uneven distribution of forces during bond strength or micro tensile testing.

CONCLUSION

It can be concluded that the use of ozonated water as a cavity cleaning solution did not influence the results of bond strength to bovine dentin.

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CONFLICTS OF INTERESTS

The authors declare no conflicts of interest.

*CORRESPONDING AUTHOR

Poliana Maria de Faveri Cardoso, Unioeste – Universidade Estadual do Oeste do Paraná, Centro de Ciências Biológicas e da Saúde, Curso de Odontologia, Rua Universitária, 2069, 85819-110 Cascavel - PR, Brasil, e-mail: polif1704@gmail.com

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