Original Research Article

Evaluation of Bond Strength of a Bioactive Adhesive System Associated with Ozonized Water on Enamel Preconditioned withPhosphoric Acid

ABSTRACT

Aims: To evaluate the effectiveness of ozonated water as a cavity cleaning solution in combination with a bioactive adhesive system on bond strength to enamel, compared to distilled water, after different storage times.

Study Design: Experimental in vitro study.

Place and Duration of Study: Department of Restorative Dentistry, State University of West Parana, Cascavel, Parana, Brazil, between September 2022 and August 2023.

Methodology: Bovine anterior teeth were divided into four groups: AD24h (distilled water and 24-hour storage), AO24h (ozonated water and 24-hour storage), AD30D (distilled water and 30-day storage), and AO30D (ozonated water and 30-day storage). After cavity cleaning, a bioactive adhesive system and composite resin were applied. Three bioactive composite resin cylinders were light-cured on each tooth using a LED light device. Microshear bond strength was tested with a testing machine (EMIC) at a speed of 1 mm/min using a 50N load cell. Statistical analysis was performed using the non-parametric Wilcoxon test ($p < 0.05$).

Results: Ozonated water significantly increased bond strength to enamel after 24 hours ($p < 0.05$). However, after 30 days, no significant differences in bond strength were observed between ozonated water and distilled water groups.

Conclusion: Ozonated water enhances initial bond strength to enamel but its effect diminishes over time. These findings suggest the need for improved adhesion techniques to achieve long-term durability in dental restorations.

Keywords:Bond Strength, Bioactive Adhesive System, Ozonated Water, Acid Conditioning.

1. INTRODUCTION

Before the advent of adhesive systems, the success of dental restorations was directly related to mechanical retention mechanisms. The development of bonding systems, coupled with the rise of minimally invasive dental procedures, has led to restorative techniques that allow greater preservation of healthy dental structure. Consequently, these materials have become highly relevant in various clinical applications [1].

Adhesive systems are responsible for promoting the adhesion of restorative materials to dental substrates (Oliveira et al., 2010). These compounds can be classified according to the different adhesive strategies used on dental structures into three main categories: conventional adhesives, self-etching adhesives, and, more recently, universal adhesives [1].

Conventional adhesives, also known as total-etch adhesives, are characterized by the preliminary and isolated application of phosphoric acid on dental structures. In enamel, conditioning with this strong acid promotes demineralization and the consequent creation of microporosities, which are then filled by hydrophobic resin monomers contained in the adhesive [1]. This technique facilitates optimal adhesion of the material to the substrate, making the restoration in enamel durable and effective [2].

Adhesion of conventional systems to dentin, however, is hindered by the more organic composition of this substrate, the intrinsic moisture in dentinal tubules, and the presence of the smear layer, a layer of residual microparticles accumulated on dentin, which can obstruct dentinal tubules [3]. Therefore, acid conditioning in dentin must promote complete removal of the smear layer, as well as demineralization of this substrate, exposing collagen fibers and allowing hybridization. However, it is necessary to maintain dentin moisture to ensure efficient penetration of resin monomers between collagen fibers [4].

Self-etching adhesives, on the other hand, do not require phosphoric acid application as they feature an acidic primer that simultaneously acts as a conditioner. Although this adhesive system shows superior adhesion to dentin compared to conventional systems, its effectiveness in enamel is significantly reduced. In this context, the selective acid etching technique of enamel, which involves applying phosphoric acid to dental enamel, is used to promote selective demineralization of this substrate, optimizing the adhesion of self-etching adhesives [4]. Additionally, universal adhesives have a pH similar to mild and very mild self-etching adhesives, providing lower adhesion strength in enamel, and have a composition similar to adhesives that contain functional monomers that chemically bond to calcium in hydroxyapatite [1].

Furthermore, cavity cleaning is a crucial procedure for the success of restorative treatments. Removal of residual debris from cavity preparation promotes better interaction between the material and the dentin substrate, increasing bond strength and reducing microleakage of the restoration, thus prolonging its durability [5]. Among the main dentin cleaning agents used in clinical practice are 2% chlorhexidine, EDTA, and saline solution. Chlorhexidine is an extensively used oral antiseptic due to its broad antibacterial spectrum and low toxicity. Ethylenediaminetetraacetic acid (EDTA) is a chelating agent that forms a stable, soluble complex with dentin calcium, making it useful in removing the smear layer [6]. In this context, the use of ozonated water for dentin cleaning is an emerging strategy in dentistry. The performance of this solution compared to traditional cleaning agents shows similar results in bond strength with adhesive systems [7].

The recent advent of bioactive adhesives represents an interesting alternative for increasing the longevity of dental restorations. These materials have the ability to neutralize metalloproteinases, enzymes involved in the degradation of collagen fibers in the hybrid layer. Thus, bioactive adhesive systems biologically modify the dentin structure, promoting remineralization of the substrate and controlling microbial growth, enhancing the stability of the adhesive interface [8].

In conclusion, the clinical relevance of adhesive systems in dental practice is clear. However, despite correct methodology, the use of these materials presents intrinsic limitations [7]. Therefore, the rising use of ozonated water as a dentin cleaning agent and the development of bioactive resins present potential alternatives to improve bonding interfaces, extending the longevity of restorative treatments. Thus, the hypothesis of this project is that the use of a bioactive adhesive system associated with ozonated water may yield superior results compared to conventional systems when applied to enamel with prior phosphoric acid conditioning. Therefore, the aim of this study is to evaluate the bond strength using microshear testing of a bioactive adhesive system associated with ozonated water in enamel conditioned with phosphoric acid.

2. METHODOLOGY

2.1 Sample Size Calculation

Using a randomized design with the Bioestat 5.3 software, a minimum sample size of 6 teeth per group was determined, considering a power analysis of 90% and a standard error of 0.01. To ensure result reliability, a total of 40 teeth were randomly distributed among four groups based on the cleaning solution.

2.2 Sample Preparation

Forty bovine anterior teeth, sourced from a local slaughterhouse, were freshly extracted and sectioned with high-speed rotation and abundant refrigeration using a diamond tip, No. 4138 (KG Sorensen), separating the crowns from the roots. The crowns were fixed in standardized PVC tubes with a diameter of 25 mm and a height of 15 mm using acrylic resin, leaving the vestibular surface exposed. The materials used were:

Table 1. Descripiton[1] and Composition of Materials Used

Material	Composition	Method of use
FL Bond II Adhesive – SHOFU	Two-step self-etch adhesive system, consisting of an acidic primer and adhesive.	Step 1: Apply the primer carefully to the dentin and enamel surfaces. Allow it to sit for 10 seconds and dry with an oil-free air jet for 5 seconds (no rinsing). Step 2: Apply a homogeneous layer of the adhesive over the entire surface. Light-cure for 10 seconds with a halogen light or 5 seconds with LED.
Beautifil Bulk Flowable Composite – SHOFU	Bulk injectable composite resin with Giomer bioactive technology.	Apply in 2 mm layers and light-cure for 20 seconds with LED.

2.3 Preparation of Ozonated Water

Ozonated water at 4 ppm was prepared at room temperature (25ºC ± 1.0ºC) and used within 5 minutes ± 1.0 minute of preparation, using an ozone generator (Ozone&Life®/O&L3.0RM, São José dos Campos, SP, Brazil) that uses pure oxygen from a cylinder, connected to a glass tower (1L/min). The amount of ozone in the water was measured by direct iodometric titration, as recommended by the International Ozone Association (IOA), which involves adding 50 ml of 1 N potassium iodide (KI) solution to the ozonated water. The chemical reaction results in the oxidation of KI by ozone, releasing iodine (I2), as per the equation O3 + 2 KI + H2O ↔ I2 + 2 KOH + O2. To ensure iodine production, it was necessary to acidify the solution by adding 2.5 mL of 1 N sulfuric acid (H2SO4) to the KI solution. The titration was then performed with 0.01 N sodium thiosulfate (Na2S2O3) until the yellow color of iodine was barely perceptible. Subsequently, 1 mL of 1% starch solution was added, and titration was continued until the blue color of the solution disappeared.

2.4 Adhesive Procedure

The FL Bond II Adhesive System (SHOFU Dental ASIA-Pacific Pte. Ltd., Kyoto, Kyoto, Japan) was used for all groups according to the manufacturer's instructions. Three composite resin cylinders were then created on the crowns of each sample. A Tygon matrix (Tygon tubing, TYG-030, Saint-Gobain Performance Plastics, Miami Lakes, FL, USA) with an internal diameter of 2 mm and a height of 2 mm was used. The matrix was positioned with the help of a clinical tweezer on the surface, and its interior was filled with Beautifil Flow Plus - F03 Composite Resin (SHOFU Dental ASIA-Pacific Pte. Ltd., Kyoto, Kyoto, Japan) in a single increment using a spatula (Thompson No. 6). Light-curing was performed with a Valo Cordless light unit (Ultradent, Indaiatuba, São Paulo, Brazil) at 1200 mW/cm^2 for 30 seconds.

2.5 Storage

The specimens were stored for 24 hours and 30 days at 37ºC in saline solution in a bacteriological incubator. The matrix was then removed using a No. 11 scalpel blade, and microshear bond strength testing was conducted.

2.6 Microshear Testing

The samples were subjected to microshear bond strength testing using a universal testing machine (EMIC DL200MF, São José dos Pinhais, Paraná, Brazil) at a speed of 1 mm/min with a 50 N load cell. The maximum force applied to the base of the cylinders was 45 N, corresponding to 10% less than the load cell's value. The microshear bond strength was evaluated by dividing the force applied at the moment of failure by the adhesive area. The data obtained were expressed in Newtons and then converted to MegaPascals (MPa). Post-test, the maximum load values supported by the composite resin-enamel interface were used for statistical analysis.

2.7 Statistical Analysis

The obtained results were tabulated and subjected to statistical analysis using Jamovi software, version 1.2.24. Initially, the data were tested for normality using the Shapiro-Wilk test, which indicated a non-normal distribution. Subsequently, statistical tests were performed to assess the presence of statistically significant differences between groups using the Wilcoxon test ($p < 0.05$).

2.8 Fracture Pattern Analysis

The fractured composite resin-enamel interface was examined under a stereomicroscope with 100x magnification (Olympus SZ40, Shinjuku, Tokyo, Japan). The analysis of the fractures revealed three distinct types of failures. Adhesive failures (A) were characterized by the detachment occurring at the composite resin-enamel interface. Mixed (M) failures involved failures at the composite resin-adhesive-enamel interface, which included both adhesive and cohesive failures. Cohesive (C) failures were those occurring exclusively within the composite resin itself.

3. RESULTS AND DISCUSSION

3.1 Bond Strength Evaluation

The obtained results were subjected to statistical analysis using the non-parametric Wilcoxon test ($p < 0.05$). Generally, the use of ozonated water resulted in higher fracture resistance when subjected to the micro-shear bond strength test at the 24-hour period compared to distilled water. However, no statistically significant difference was observed between the groups in the 30-day period.

Furthermore, an intra-group analysis showed no significant differences within the ozonated water group. In contrast, for the distilled water group, a significant difference was found in the middle third between the 30-day analysis and all 24-hour analyses, as well as in the incisal third at the 30-day analysis. Detailed results are presented in Table 2.The fractured composite resin-enamel interface was examined under a stereomicroscope with 100x magnification (Olympus SZ40, Shinjuku, Tokyo, Japan). The analysis of the fractures revealed three distinct types of failures. Adhesive failures (A) were characterized by the detachment occurring at the composite resin-enamel interface. Mixed (M) failures involved failures at the composite resin-adhesive-enamel interface, which included both adhesive and cohesive failures. Cohesive (C) failures were those occurring exclusively within the composite resin itself.

Table 2. Median values and interquartile range of micro-shear bond strength (N) over time, comparing the use of ozonated water and distilled water for cavity cleaning on enamel.

Time		**24 hours**			**30 days**	
Dental third	Cervical	Middle	Incisal	Cervical	Middle	Incisal
Distilled Water	5.71 (± 2.26) Aa	5.71 (± 4.33) Aa	3.81 (± 3.61) Aa	11.1 (± 2.4) Aab	11.6 (± 7.12) Ab	8.91 (± 9.66) Aa
Ozonated Water	11.3 (± 11.05) Ba	15.8 (± 12.85) Ba	11.7 (± 7.44) Ba	14.7 (± 8.8) Aa	10.9 (± 4.8) Aa	10.6 (± 4.57) Aa

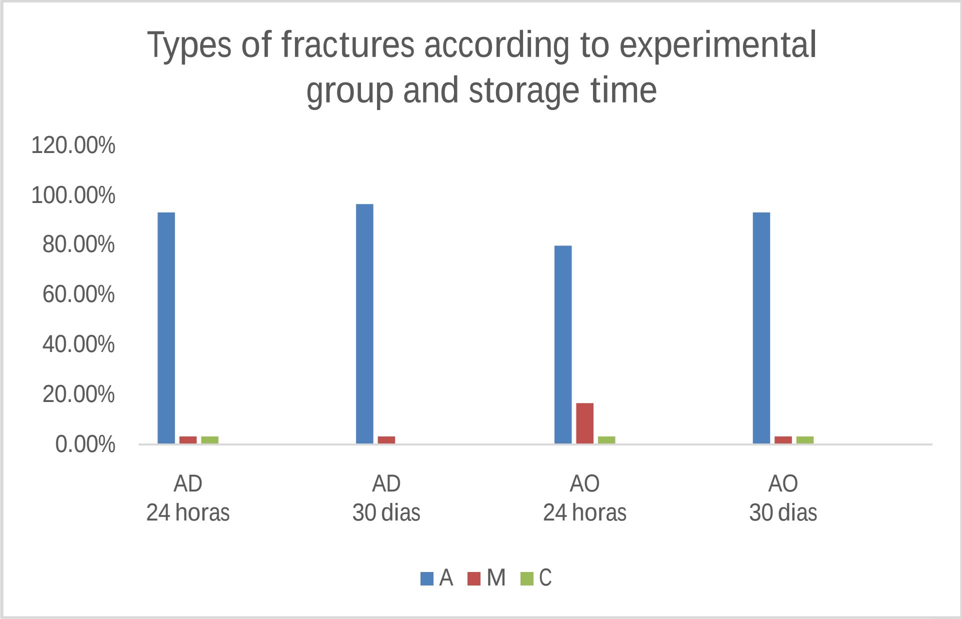
**Different lowercase letters within the same row indicate significant differences with $P < 0.05$ in intra-group analysis using the Wilcoxon test.*

***Different uppercase letters in the same column indicate significant differences with $P < 0.05$ in inter-group analysis using the Wilcoxon test.*

3.2 Fracture Pattern Evaluation

A higher incidence of adhesive fractures was observed, followed by mixed and cohesive fractures. Figure 1 illustrates the types of fractures for each experimental group, considering the storage time.

Figure 1. Graph of fracture types based on the experimental group and storage time.



3.3 Discussion

Cavity cleaning solutions play a crucial role in the success of restorative treatment by removing debris from the tooth preparation, enhancing the adhesion of the restorative material to the tooth, and reducing microleakage of the restoration [5]. Common cavity cleaning agents in clinical practice include 2% chlorhexidine, EDTA, and saline solution. Recently, ozonated water has gained attention for its strong antibacterial action, which plays a significant role in preventing secondary carious lesions and enhancing the longevity of adhesive restorations [9]. Additionally, ozonated water has significant analgesic properties, reducing postoperative sensitivity and providing additional benefits over other cavity cleaning materials. However, recent studies indicate that despite these additional properties, ozonated water does not negatively influence the bond strength of adhesive restorations. Its performance in terms of bond strength is similar to that of traditional cleaning agents [9].

The analysis of the obtained results shows that the use of ozonated water provided significantly higher bond strength when subjected to the micro-shear bond strength test at the 24-hour period compared to distilled water. This finding suggests that ozone may have a positive effect on the initial adhesion of bioactive composite resins to dental enamel. The increase in bond strength could be attributed to the antimicrobial and oxidative properties of ozone, which may facilitate more effective cleaning of the tooth surface and thus improve the interaction between the adhesive and enamel. Moreover, the adhesive system used promotes bonding to the tooth structure through chelation with the calcium present on its surface. Therefore, the calcium exposure provided by cavity cleaning with ozonated water may have favored the formation of a more resistant adhesive interface, contributing to the superior results observed at the initial 24 hours. However, it is noteworthy that extending the storage period to 30 days did not reveal any statistically significant difference between the groups. This result corroborates findings by Celiberti et al. (2006), where the use of ozonated water as a cavity cleaning solution did not affect fracture resistance over time. Thus, the results suggest that the beneficial influence of ozone on bond strength may be more pronounced in the initial stages of adhesion but may diminish over time. This decrease could be due to degradation of adhesive components or reactivation of chemical interactions between the adhesive and the tooth substrate.

Additionally, intra-group analysis revealed a significant difference in the middle third between the 24-hour and 30-day analyses, as well as in the incisal third at the 30-day analysis in the distilled water group. This difference may indicate a variation in bond strength of the bioactive resin over time, specifically in the distilled water group. The variation in bond strength could be attributed to factors such as hydrolytic degradation of the adhesive or changes in the mechanical properties of the resin over time.

The data suggest that selecting ozonated water as a cleaning agent during the adhesion procedure may play a critical role in the initial bond strength of bioactive composite resins, with potential influence on restoration longevity. Furthermore, the antibacterial effect of ozonated water is noteworthy, as it significantly contributes to the reduction of recurrent dental caries. This effect can be attributed to the oxidation potential of ozone, capable of damaging the bacterial cell wall and cytoplasmic membrane.

Regarding fracture types, adhesive fractures were predominant in all groups, followed by mixed and cohesive failures. The results of the fracture pattern evaluation provide insight into the response of the tested materials to the use of distilled water and ozonated water, as well as to the storage time. The higher incidence of adhesive fractures is relevant as it suggests that material adhesion may be subject to improvements, considering the need for effective adhesion in dental applications. Additionally, the predominance of adhesive fractures indicates that the adhesive interface remains the most vulnerable area in the restoration, highlighting the importance of developing techniques and materials that can enhance this adhesion.[2]

Although the results provide valuable information on the efficacy of ozonated water as a cavity cleaning agent on the bond strength of a bioactive adhesive system, this study has limitations that should be considered. The experiment was conducted under controlled laboratory conditions, which may not account for the potential variabilities of clinical practice. Additionally, the bond strength and fracture pattern analyses were limited to 24-hour and 30-day storage periods, not allowing for an assessment of results over extended periods. Therefore, further studies are needed to evaluate the longevity of these restorations over longer periods and under variable clinical conditions. This will contribute to the development of more effective solutions for adhesive dental applications.

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