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Abstract

The aim of this study was to investigate the potential of ozonated water as an oxidative cleaning solution to improve the bond strength of a bioactive adhesive system to enamel without prior etching. Forty bovine anterior tooth crowns were used and divided into four groups according to the cleaning solution and storage time: AD24h (cleaning with distilled water, 24 hours of storage), AO 24h (cleaning with ozonized water, 24 hours of storage), AD30D (cleaning with distilled water, 30 days of storage) and AO 30D (cleaning with ozonized water, 30 days of storage). All groups were subjected to the application of the bioactive adhesive system, followed by the manufacture of bioactive bulk fill flow resin composite cylinders (2x2 mm) on the crowns. After each storage period, the samples

were evaluated using microshear tests and fracture analysis. The data was statistically analyzed using JAMOVI software, version 1.2.24. The results indicated that there was no statistically significant difference in most of the tooth thirds and groups evaluated, except in the cervical third after 24 hours, where ozonated water showed greater bond strength. This study suggests that ozonized water can potentially benefit the adhesion of bioactive adhesive systems to dental enamel, especially under specific storage and application conditions.

Keywords: Dental enamel. Ozone. Composite resin. Dentistry.

Introduction

The evolution of dental materials and their properties is becoming increasingly evident in dentistry. The quality of selecting a good adhesive system and the correct restorative technique allows the resin composite to be more effective, durable and functional ¹. In order to restore lost natural aesthetics, some various restorative materials and techniques enable long-term clinical success ².

Resin composite is a restorative material widely used in restorative dentistry, as it provides conservative, relatively low-cost treatment with good aesthetic purposes ³. However, expectations about these materials leave a lot of room for advances in their mechanical properties, such as failures caused by polymerization-induced stress, polymerization contraction and thermal expansion mismatch, abrasion and wear resistance, toxicity and marginal infiltration ^{4, 5}.

Biomaterials were developed to improve the interaction between the restorative material and the dental structure ⁶. The concept behind the development of bioactive restorative materials aims not only to repair lesions in dental hard tissues, but also to incorporate biological properties that prevent the growth of secondary caries, without adversely affecting the host cells ⁷. Biocompatibility is based on a material's ability to interact properly with biological structures, without causing a toxic or immunological reaction ⁸.

Studies on the initial properties of giomers have reported initial mechanical properties that are comparable to those of a resin composite ⁹. Gioner has been described as an "intelligent particle", that emerged intending to combine the esthetics and strength of resin composite with the fluoride-releasing capacity of glass ionomer¹⁰.

This material uses glass ionomer technology with a pre-activated S-PRG surface ¹¹. When in contact with polyacrylic acid, the fluoraminosilicate particles react and are incorporated into the resin, resulting in a continuous release of fluoride. This happens as soon as it interacts with saliva from the oral environment ¹²⁻¹⁴. In addition to hindering bacterial adhesion to the tooth ¹⁵, the ions act as a buffer, neutralizing the acids produced by these microorganisms ¹¹.

Therefore, cavity cleaning is essential to maximize adhesive system properties. Ozone is a molecule made up of three oxygen atoms, which form naturally. It protects the Earth's surface and human life from harmful ultraviolet rays ¹⁶. Ozone therapy is a biologically beneficial agent in which oxygen/ozone is supplied as a gas or dissolved in a water or oil base for medicinal purposes, which has therapeutic uses in various dental treatments. When used in conjunction with other treatments, ozone therapy has many benefits¹⁷. Due to this, ozonated water has been proposed as a method of cavity disinfection before restorative procedures ¹⁸. It has shown several advantages, due to its antioxidant, healing and remineralizing properties, and can contribute to increasing bond strength to dental substrates ¹⁹⁻²¹. This study aimed to verify whether using an oxidative cleaning solution, such as ozonized water, can improve the bond strength of a bioactive adhesive system on enamel without prior conditioning.

Methodology

Sample preparation

Forty bovine anterior teeth from a local slaughterhouse were selected, freshly extracted and sectioned at high speed with abundant cooling using a No. 4138 diamond tip (KG Sorensen), separating the crowns from the roots. The crowns were fixed in standardized PVC tubes with acrylic resin, leaving the vestibular surface free. They were then divided into 4 groups:

Group 1: AD 24h - Cavity cleaning with distilled water and storage for 24 hours;

Group 2: AO 24h - Cavity cleaning with ozonized water and storage for 24 hours;

Group 3: AD 30D - Cavity cleaning with distilled water and storage for 30 days;

Group 4: AO 30D- Cavity cleaning with ozonized water and storage for 30 days.

The materials used in each group are shown in Table 1.

Table 1 - Description and composition of the materials used.

Material	Composition	Manufacturer	Mode of use
FL-Bond II – Two-step, self-etching adhesive system.	Primer: Carboxylic acid monomer, phosphinic acid monomer, 6-MHPA, water, solvent and photoinitiator. Adhesive: HEMA, UDMA, TEGDMA, 40% S-PRG-filler particles, photoinitiator.	Shofu, Kyoto, Japan.	Step 1: Dispense the Primer and carefully apply it to the dentin and enamel surfaces, leave for 10 seconds, dry with an oil-free air jet for 5 seconds (Do not rinse) Step 2: Dispense the Bonding Agent and apply a homogeneous layer over the entire surface, light-cure for 10 seconds with halogen light or 5 seconds with LED.
Beautiful Flow Plus F00	Base resin: Bis-GMA resin (15% by weight) /TEGDMA (13% by weight). Filler: Multifunctional glass filler and S-PRG filler based on fluoroaluminosilicate glass. DL-Camforquinone	Shofu, Kyoto, Japan.	In the case of a deep cavity, proceed in several stages (application/curing) without exceeding 4 mm in thickness at a time. Light cure for 10 seconds with LED light.

*Bis-GMA: bisphenol-A-diglycidyl methacrylate; TEGDMA: triethylene glycol dimethacrylate; 6-MHPA: 6-methacryloxyhexyl 3-phosphonoacetate; HEMA: 2-hydroxyethyl methacrylate; UDMA: urethane dimethacrylate; S-PRG filler: glass ionomer filler pre-reacted on the surface.

Preparation of ozonated water

The 4 ppm⁷ ozonated water was prepared at a room temperature of 25°C ± 1.0 °C and 5 minutes ± 1.0 min before use and used up to 5 minutes ± 1.0 min after preparation, using an ozone generator (Ozone & Life® /O&L3.0RM, São José dos Campos, SP, Brazil) which uses pure oxygen from a cylinder attached to a glass tower (1L/min). The amount of ozone in the water will be measured using direct iodometric titration, as recommended by the International Ozone Association (IOA), which consists of adding 50 ml of potassium iodide solution (KI) 1 N, in previously ozonized water. The chemical reaction that takes place in this procedure results in the oxidation of KI by ozone, promoting the release of iodine (I₂), according to the equation $O_3 + 2 KI + H_2O \leftrightarrow I_2 + 2 KOH + O_2$. In order to ensure the production of I₂, it will be necessary to acidify the medium by adding 2.5 mL of 1 N sulfuric acid (H₂SO₄) to the KI solution. Then titrate with 0.01 N sodium thiosulfate (Na₂S₂O₃) until the yellowish color of the iodine becomes barely perceptible. Then 1 mL of 1% starch indicator solution is added and the titration is resumed until the blue color of the solution disappears.

Bonding procedure

Next, the FL Bond 2- (SHOFU, Kyoto, Japan) adhesive system was applied to all groups according to the manufacturer's instructions. Three composite resin cylinders were then made in the crowns of each group. A Tygon matrix (Tygon tubing, TYG-030, Saint-Gobain Performance Plastic, Maime Lakes, FL, USA) with an internal diameter of 2 mm and a height of 2 mm was used. The matrix was positioned with the aid of clinical forceps on the surface. Then its interior was filled with Beautifil Flow Plus - F03 composite resin (SHOFU, Kyoto, Japan) in a single increment. Following the manufacturer's recommendations, photoactivation was carried out using a Valo light device (Ultradent Products, South Jordan, UT, USA).

Storage

The specimens were stored for 24 hours and 30 days at 37°C in saline solution in a bacteriological oven, after which the matrix was removed with a No. 11 scalpel blade and the microshear test was carried out.

Microshear test

The samples were subjected to the micro-shear test on a universal testing machine (EMIC) 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 was transformed into MPa and submitted to statistical analysis.

Fracture analysis

The fractured resin-enamel/dentin interface was analyzed under a stereoscopic loupe at 100x magnification (Olympus SZ40, Japan). The types of failure were classified as:

Adhesive (A): failure at the composite resin-dentin/enamel interface.

Mixed (M): failures at the adhesive/enamel-dentin/composite resin interface, including cohesive failures.

Cohesive in Composite Resin (CC): failure exclusively in composite resin.

Cohesive in Dentin (CD): failure exclusively within dentin/enamel.

Statistical analysis

The results were tabulated and subjected to statistical analysis using JAMOV software, version 1.2.24. Initially, the data was assessed for the requirement of normal distribution using the Shapiro Wilk test, with a negative result. After analyzing this prerequisite, statistical tests were carried out to assess the existence of statistically significant differences between the groups using the Wilcoxon test ($p < 0.05$).

Results

The results obtained were statistically analyzed using the non-parametric Wilcoxon test ($p < 0.05$).

In general, using ozonated water promoted greater resistance to fracture when subjected to the microshear test for the cervical third at 24 hours compared to using

distilled water. For the other thirds, there was no statistically significant difference between the groups at both times.⁴

In turn, for the intra-group analysis, there was a statistically significant difference for ozonized water between the cervical third in the 24-hour period and the same third in the 30-day period and with the incisal third in the 30-day period, while for distilled water it was possible to observe a difference between the cervical and incisal thirds in the 30-day analysis. The data is shown in Table 2.

Table 2 – Median values and interquartile deviation of micro-shear bond strength (N), according to time, when using ozonized water and distilled water for cavity cleaning before using self-adhesive resin.

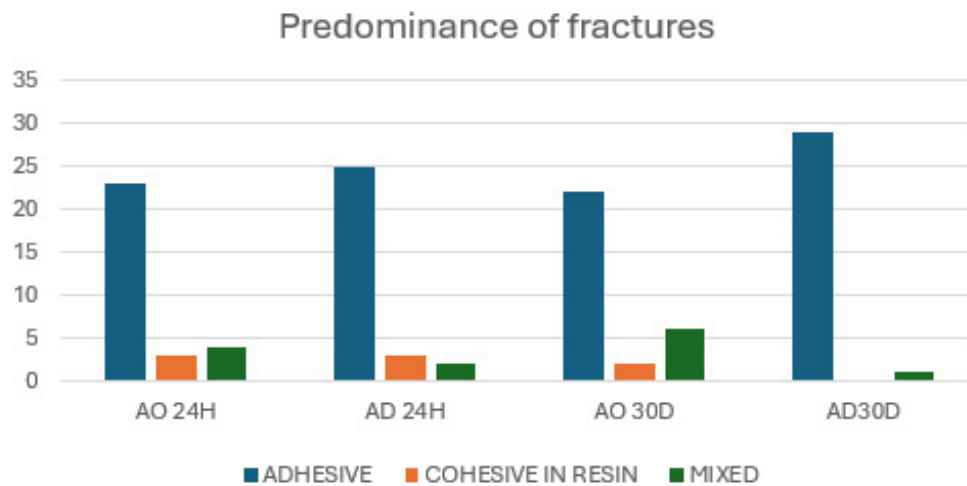
Time	24 hours			30 days		
	Cervical	Medial	Incisal	Cervical	Medial	Incisal
Distilled water	2.93 (+ 1.74) Aab	2.37 (+ 1.76) Aab	2.77 (+ 2.52) Aab	8.44 (+ 8.09) Aa	7.9 (+ 9.19) Aab	4.94 (+ 9.66) Ab
Ozonated water	8.31 (+ 2.16) Ba	8.4 (+ 0.76) Aab	8.91 (+ 3.31) Aab	7.42 (+ 2.39) Ab	6.78 (+ 3.49) Aab	6.79 (+ 4.33) Ab

*Different lowercase letters in the row = significant differences with $p < 0.05$ in the intra-group analysis using the Wicoxon test.

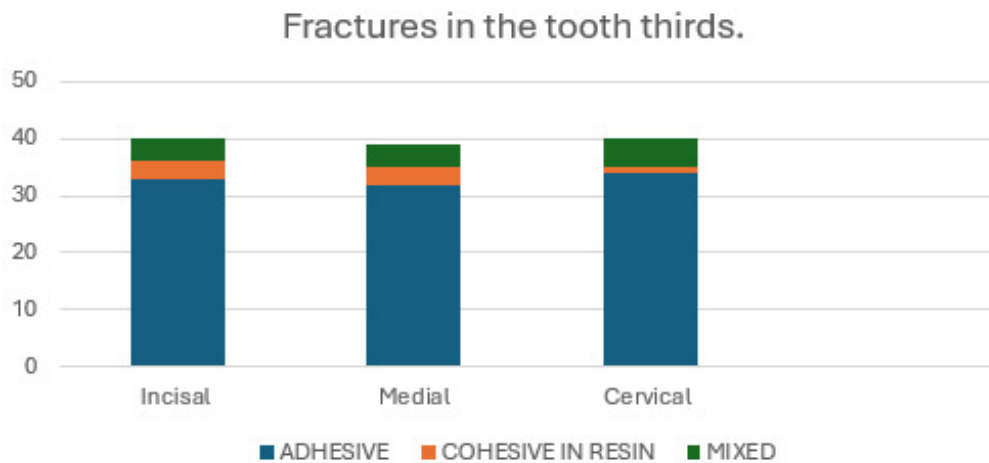
**Different capital letters in the column = significant differences with $p < 0.05$ in the inter-group analysis using the Wicoxon test.

Regarding the classification of the type of fracture, the predominant fracture was adhesive, with a significant difference when compared to the others. The second most common type was mixed, followed by cohesive in resin. The type of cohesive fracture in dentin was not observed during the analysis. Data on the types of fracture in each group is shown in Graph 1.

Graph 1 – Predominance of fractures.



Graph 2- Types of fractures in tooth thirds.



Discussion

Ozone acts as an oxidizer, which has led to its use in the medical and dental fields. Ozone has unique properties, which is why it has a wide range of applications. These properties include antimicrobial, immune stimulating, antihypoxic, vasodilating, detoxifying and biosynthetic. In addition, ozone is a powerful repair agent for pulp tissue and is completely biocompatible ²². Despite having interesting properties for its use, some studies suggest that ozonized water may interfere with the bond strength of long-term restorations ²³.

Confirming part of the hypothesis of this study, ozonated water as a cavity-cleaning solution improved enamel bond strength in the cervical third during the 24-hour storage period. However, in other dental thirds and at other storage times, no significant differences were observed. Regarding the use of ozonized water as a cavity cleaning agent, these findings corroborate almost entirely with the studies by Detogni, et al. ²⁴

(2023) and Cardoso, et al. ²⁵ (2023) who observed that the use of ozonized water did not interfere with the bond strength of bulk fill flow resins. According to Cangul, et al. ²⁶ (2020), the use of ozone before the restorative procedure significantly increased the bond strength of adhesive agents. These findings may be promising, as they contradict the results of the study by Bilgili, et al. ²⁷ (2022), which revealed that the use of ozone decreased the bond strength values of a universal adhesive in dentin, justifying that this difference could be explained due to the presence of oxygen residues after the use of ozone that would affect polymerization. These differences in results can be explained by the methodological differences used in the studies, such as the form of ozone application (gas, ozonated water or oil) and the difference in concentration.

Another factor that may have corroborated the higher bond strength values in enamel in the cervical region is the use of the FL-Bond II adhesive system (Shofu, Kyoto, Japan), since the composition of this self-etching adhesive contains monomers that are able to chemically and mechanically bond the adhesive interface to the dental substrate. Li, et al. ²⁸ (2019) demonstrated that the addition of dimethylaminohexadecyl methacrylate (DMAHDM) and amorphous calcium phosphate particles, a biomaterial, promoted antibacterial activity and increased interfacial durability in adhesive systems.

Regarding bond strength values, the smaller internal diameter and height of the Tygon matrix may have influenced the results. Since this size reduction may have influenced the force exerted on the load cell, resulting in the lower values found ²⁹.

As for the types of fractures observed after the microshear test, there was a predominance of adhesive fractures in both groups and all tooth thirds, followed by mixed and cohesive resin fractures; in this study, no type of cohesive resin fracture was observed. By remaining on the surface of dental tissues, oxidizing substances such as ozone, hydrogen peroxide and carbamide peroxide can negatively affect the monomer chain that grows during adhesive polymerization ^{30 - 32}. The studies by Qeblawi et al. ³³ (2015) and Ahn et al. ³⁴ (2020) suggest that, in the shear strength test, the distribution of stresses in the bond area and the differences in the elastic properties of the materials can lead to significant changes in fracture patterns.

However to a lesser extent, resin cohesive failures were also found in this study, especially in the incisal and middle thirds. The oxidizing action of ozone can interfere with the prismatic structure of the dental substrate, so the neutralization of acidic monomers is reduced and the residual acidity affects the setting reaction and, consequently, the polymerization of the materials ³⁵. The non-neutralization of monomers

increases water sorption, solubility and hygroscopic expansion stress ³⁶. Factors that may explain the pattern of cohesive fractures in resin.

Given the limitations of this study, further laboratory tests and clinical trials are therefore necessary to confirm the data obtained in this *in vitro* study. Further studies on the use of ozonated water in dentistry are needed to prove its efficacy and to determine protocols for using ozone.

Conclusion

It can be concluded that ozonized water obtained the best bond strength results on enamel in the cervical third during the 24-hour storage period. In the other storage periods and tooth thirds, there were no significant differences in enamel bond strength.

Author's contribution

Author 1: project administration, writing and review, investigation, Author 2: writing and editing, Author 3: resources, Author 4: supervision, Author 5: conceptualization, original draft, supervision.

Conflicts of interests

All authors have read the final version of the manuscript and declare that there is no conflict of interest.

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