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Research Article

Effect of Ozonated Water on the Bond Strength of FL Bond II Adhesive to Enamel

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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: AD24 h (cleaning with distilled water, 24 h of storage), AO 24 h (cleaning with ozonized water, 24 h of storage), AD30D (cleaning with distilled water, 30 days of storage) and AO 30D (cleaning with ozonized water, 30 days of storage) All groups received the application of the bioactive adhesive system, followed by the fabrication of 2×2 mm bioactive bulk fill flow resin composite cylinders on enamel. After each storage period, the samples were evaluated using microshear test 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 h, where ozonated water showed higher bond strength. This study indicates that ozonated water may enhance the adhesion of bioactive adhesive systems to dental enamel, particularly when specific storage and application conditions are optimized.

Keywords: Enamel, Resin Composite, Ozone, Microshear

Introduction

Advancements in dental materials and their properties are becoming increasingly evident in the field of modern dentistry. The selection of an effective adhesive system and the correct restorative technique allow resin composites to be more durable, functional, and efficient (Watts, 2020). To restore natural aesthetics, various restorative materials and techniques facilitate long-term clinical success (Spezzia, 2019). Resin-based composites have become a common choice in restorative dentistry, primarily because they allow for minimally invasive procedures, offer favorable esthetic results, are economically accessible, and have good aesthetic outcomes (Barcelos *et al.*, 2020). However, there remains significant room for improvement in their mechanical properties, which still present challenges such as stress from polymerization shrinkage, incompatibility in thermal expansion, limited wear resistance, potential discoloration over time, cytotoxic effects, and marginal infiltration (Boaro *et al.*, 2019; Dias *et al.*, 2018).

Biomaterials were developed to improve the interaction between restorative materials and dental structures (Lalzawmliana *et al.*, 2020). The advancement of bioactive restorative materials aims not only to restore lesions in hard dental tissues but also to incorporate biological functionalities capable of preventing secondary caries without adversely affecting host cells (Wuersching *et al.*, 2023). Biocompatibility refers to a material's ability to interact with biological structures without causing toxic or immunological reactions (Kus-Liśkiewicz & Fickers, 2021).

Preliminary studies on giomers have shown that their mechanical properties are comparable to those of conventional resin composites (Tsujimoto *et al.*, 2017). Giomers are often referred to as "smart materials" due to their ability to merge the mechanical strength and esthetic performance of resin composites with the fluoride-releasing capacity of glass ionomers (Rusnac *et al.*, 2019). This material incorporates glass ionomer technology with a pre-activated S-PRG surface (Moecke *et al.*, 2020). When exposed to polyacrylic acid, the fluoraminosilicate particles react and are incorporated into the resin, resulting in a continuous release of fluoride, which occurs upon interaction with saliva in the oral environment (Colceriu Burtea *et al.*, 2019; Francois *et al.*, 2020; Harhash *et al.*, 2017). Additionally, these

ions hinder bacterial adhesion to the tooth (Shimizubata *et al.*, 2020) and act as a buffer to neutralize acids produced by microorganisms (Moecke *et al.*, 2020).

To maximize the properties of adhesive systems, cavity cleaning is a critical step. Ozone, a molecule composed of three oxygen atoms, naturally forms in the environment and protects the Earth's surface and life from harmful ultraviolet rays (Domb, 2014) Ozone therapy is considered a biologically beneficial approach, where ozone or oxygen/ozone mixtures are delivered in gaseous form or dissolved in water or oils for therapeutic use. In dentistry, its application either alone or combined with other treatments has demonstrated multiple clinical benefits (Lubojanski *et al.*, 2021). As a result, ozonated water has been suggested as an effective agent for cavity disinfection prior to restorative procedures. Its antioxidant, regenerative, and remineralizing effects have been associated with improved adhesion to dental substrates (Coelho *et al.*, 2020; Hubbezoğlu & Oğuzhan, 2018; Yeşilöz Gökçen *et al.*, 2019; Cangul *et al.*, 2019).

This study stands out by investigating the use of ozonated water as a cleaning solution to enhance the adhesion of bioactive adhesive systems to enamel without prior conditioning. Its novelty lies in leveraging ozonization to improve adhesion by exploring its antioxidant, healing, and remineralizing properties.

Furthermore, by addressing ozonization as a pre-restorative procedure, the study fills gaps in the literature related to optimal protocols and concentrations of ozonated water. Since there are only a few studies, each with significant methodological differences, there is potential for contradictory and conflicting results. This innovative approach may offer a more effective alternative for enhancing the durability and strength of dental restorations, representing a significant advancement in clinical practice and providing long-term benefits for restorative treatment success.

The aim of this study was to determine whether ozonated water, as an oxidative cleaning solution, could improve the bond strength of a bioactive adhesive system on enamel without prior conditioning.

Materials and Methods

Sample Preparation

Given the difficulties in working with human teeth, bovine teeth were chosen for this study, as supported by the research of de Carvalho *et al.* (2018) and Naik *et al.* (2016). Their findings indicated no significant differences in bond strength (both tensile and shear) between bovine and human teeth, whether enamel or dentin regardless of whether the teeth were permanent or deciduous. Additionally, bovine enamel was found to be the most similar to human enamel in terms of chemical composition (Macedo *et al.*, 2021), enamel thickness (de Carvalho *et al.*, 2018), and resistance to acid (Kumari *et al.*, 2023), when compared to enamel from other mammalian species.

For this experiment, forty freshly extracted bovine anterior teeth were sourced from a local slaughterhouse. These teeth were sectioned at high speed with abundant cooling using a No. 4138 diamond tip (KG Sorensen), separating the crowns from the roots. The crowns were then fixed in standardized PVC tubes with acrylic resin, leaving the vestibular surface exposed. The teeth were subsequently divided into four groups: Group 1: AD 24h – Cavity cleaning with distilled water and storage for 24 h. Group 2: AO 24 h Cavity cleaning with ozonated water and storage for 24 h. Group 3: AD 30D–Cavity cleaning with distilled water and storage for 30 days. Group 4: AO 30D–Cavity cleaning with ozonated water and storage for 30 days. The materials used in each group are detailed in Table (1).

Table 1: Description of the materials

Material	Composition	Manufacturer	Mode of use
FL-Bond II	Primer: MHPA, water, solvent, photoinitiator. Adhesive: HEMA, UDMA, TEGDMA, 40% S-PRG filler, photoinitiator	Shofu, Kyoto, Japan	Apply primer, wait for 10s, and dry with oil-free air for 5s (no rinse). Apply bonding agent and light-cure (10s halogen/5s LED)
Beautifil Flow Plus F00	BisGMA/TEGDMA resin (15%/13%), 67.3% filler (glass & S-PRG), 0.01–4.0 µm particles (avg. 0.8 µm), camphorquinone photoinitiator	Shofu, Kyoto, Japan	For deep cavities, apply and cure in layers up to 4 mm, light-cure each for 10s with LED.

**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*

Sample Size Calculation

The sample size calculation was based on probability distributions of the F family, with repeated measures design and interaction within and between factors. The effect size used was 0.15, the type 1 error (α) was set at 0.05 and the analysis power was 0.85, ensuring a minimum of 108 sample units (specimens), with 9 samples per experimental group. For practical purposes, 10 samples were selected per experimental group, resulting in a total of 120 specimens. The sample size calculation was conducted using the GPower software (version 3.1.9.2, University of Düsseldorf, Düsseldorf, Germany).

Preparation of Ozonated Water

The 4 ppm7 ozonated water was prepared at a room temperature of 25±1.0ºC and 5±1.0 min before use and used up to 5±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 ozonated 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 O3+2 KI + H2O ↔ I2 + 2 KOH + O2. 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.

The 4 ppm concentration was chosen based on the findings of Kumari *et al.* (2023); and Macedo *et al.* (2021), who demonstrated that this concentration provided the highest adhesive bond strength when used for caries-affected dentin disinfection. Additionally, it was observed that the ozonated water did not alter the pH and caused modifications to the enamel surface. The ozonated water was used immediately after production to maximize its antimicrobial efficacy, the solution was freshly generated using a dedicated ozone generator and directly applied to the enamel surface for 30 seconds. This approach ensured that the ozone concentration remained at an effective level during application.

Bonding Procedure

Next, the FL Bond 2- (SHOFU, Kyoto, Japan) adhesive system was applied to all groups following the manufacturer's instructions. Three composite resin cylinders were then fabricated on the crowns of each group. 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 on the surface with the assistance of clinical forceps. The interior of the matrix was then filled with Beautiful Flow Plus - F03 composite resin (SHOFU, Kyoto, Japan) in a single increment. In accordance with the manufacturer's recommendations, photoactivation was performed using a Valo light device (Ultradent Products, South Jordan, UT, USA).

Storage

The specimens were stored for 24 h and 30 days at 37°C in a saline solution within a bacteriological oven. A temperature of 37°C is commonly used for storing bovine tooth samples in enamel analysis as it mimics the natural oral temperature. The incubator allowed for control of ambient light and the samples were kept in the dark during storage. After the storage period, the matrix was removed using a No. 11 scalpel blade, and the micro shear test was performed.

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 for 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 follows: Adhesive (A): Failure at the resin composite/dentin/enamel interface; Mixed (M): failures at the adhesive/enamel-dentin/resin composite interface, including cohesive failures; Cohesive in Composite Resin (CC): Failure exclusively in resin composite; Cohesive in Dentin (CD): Failure exclusively within dentin/enamel.

Statistical Analysis

The results were tabulated and subjected to statistical analysis using JAMOVI 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 were statistically analyzed using the non-parametric Wilcoxon test ($p<0.05$). In general, the use of ozonated water resulted in higher fracture resistance when subjected to the micro shear test for the cervical third at 24 hours compared to distilled water. For the other time periods, no statistically significant differences were observed between the groups.

In the intra-group analysis, a statistically significant difference was found for ozonated water between the cervical third at 24 hours and the same third at 30 days, as well as between the cervical third at 30 days and the incisal third at 30 days. For distilled water, a difference was observed between the cervical and incisal thirds in the 30-day analysis. The data are presented in Table (2).

Regarding the classification of fracture types, the predominant fracture was adhesive, with a significant difference observed when compared to the other types. The second most common type was mixed, followed by cohesive fractures in the resin composite. No cohesive fractures were observed in dentin during the analysis. The distribution of fracture types in each group, as well

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as their distribution across the dental thirds, is presented in Figures (1-2).

Table 2: Median values and interquartile deviation of micro-shear bond strength (N), according to time, when using ozonated water and distilled water for cavity cleaning prior to using the bioactive system; 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. ± Standard deviation

Time	24 hours			30 days		
Dental third	C	M	I	C	M	I
Dest. W	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
Oz. W	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

** Statistically significant difference founded.In these third and time better results on the bond strength. Dest. Water Distilled water. Ozo Water Ozonated Water. C Cervical. M Medial, I Incisal*

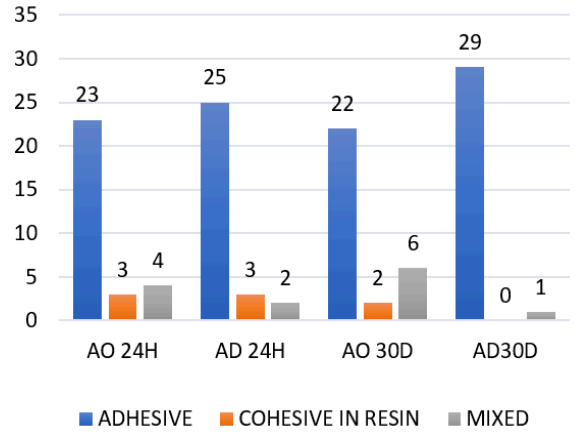

Fig. 1: Predominance of fractures

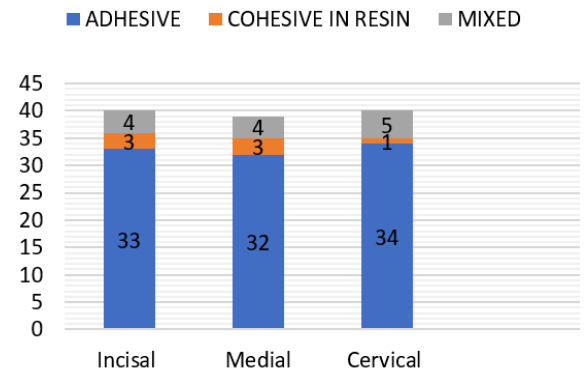

Fig. 2: Fractures in dental thirds

Discussion

Ozone functions as a powerful oxidizing agent, a property that has supported its broad application across both medical and dental disciplines. Its unique properties, including antimicrobial, immune-stimulating, antihypoxic, vasodilating, detoxifying, and biosynthetic effects, make it suitable for various applications. Additionally, ozone exhibits strong reparative potential for pulp tissue and demonstrates full biocompatibility (Alpan & Bakar, 2018). However, despite its favorable properties, some studies have indicated that ozonated water may adversely affect the long-term bond strength of adhesive restorations (Akturk *et al.*, 2019).

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, no significant differences were observed in other dental thirds or at other storage times. These results regarding the use of ozonated water for cavity cleaning align with the findings of Detogni *et al.* (2023); and Cardoso *et al.* (2023), both of whom concluded that the use of ozonated water had no adverse effect on the bond strength of bulk-fill flowable resin composites. In contrast, Cangul *et al.* (2020) reported that applying ozone prior to the restorative procedure led to a significant improvement in the bond strength of adhesive systems. These results present promising insights, as they challenge the findings of Bilgili *et al.* (2022), which reported a decrease in bond strength values for a universal adhesive on dentin when ozone was used. This discrepancy may be attributed to the presence of oxygen residues following ozone application, which could negatively impact polymerization. Additionally, Dalkilic *et al.* (2012) reported lower Bond Strength (BTS) values when using ozonated water with self-etch adhesives, linking these outcomes to variations in application methods and concentrations. These differences in results can be explained by methodological variations across studies, such as the type of substrate (enamel or dentin), the form of ozone application (gas, ozonated water, or oil), differences in ozone concentration, application time, and even the ozone-generating device used. Given that ozone is an unstable molecule that can rapidly revert to its O_2 form, the exposure time plays a crucial role and may significantly impact the clarity and reliability of the results. This suggests a need for further investigations to clarify the conditions under which ozone may be detrimental, ensuring that its application does not compromise adhesive performance in clinical practice.

The results indicate that the positive effect of ozone on bond strength is likely more significant during the early stages of adhesion, though it may decrease over time. This reduction might be due to the degradation of adhesive components or the reactivation of chemical interactions between the adhesive and the dental substrate (Moecke *et al.*, 2020).

Related to the better results in the cervical third at both time intervals, previous research has shown that enamel bond strength can vary according to the enamel

region and its microstructure, due to the complex spatial arrangement of enamel prisms and inter prisms (Shimada Tagami, 2003). Wang *et al.* (2018) reported higher bond strength values in the cervical region of bovine enamel when compared to the middle and incisal thirds. This finding could be explained by the fact that transversally cut enamel prisms are more easily etched, facilitating the penetration of the adhesive and resin (Van Meerbeek *et al.*, 2011). Moreover, the prisms in the cervical third, when observed in horizontal and tangential sections, are considerably thinner than those found in the incisal and middle thirds. Consequently, the number of transversally cut prisms per surface area is higher in the cervical third, potentially contributing to the improved bond strength observed in this region. The microstructure of enamel in the cervical third may have influenced the bond strength values observed in this study (Wang *et al.*, 2018).

Another factor that may have contributed to the higher bond strength values in the enamel of the cervical region is the use of the FL-Bond II adhesive system (Shofu, Kyoto, Japan). The composition of this self-etching adhesive includes monomers capable of both chemically and mechanically bonding the adhesive interface to the dental substrate. Li *et al.* (2019) demonstrated that the addition of Dimethylamino Hexadecyl Methacrylate (DMAHDM) and amorphous calcium phosphate particles, a biomaterial, promoted antibacterial activity and enhanced interfacial durability in adhesive systems.

In relation to the bond strength values, the reduced internal diameter and height of the Tygon matrix may have had an impact on the outcomes. The reduction in size could have affected the force exerted on the load cell, potentially leading to the lower values observed in this study (Vieira *et al.*, 2016).

As shown in Figure (1), the types of fractures observed after the micro shear test revealed a predominance of adhesive fractures in both groups and across all tooth thirds, followed by mixed and cohesive resin fractures. In this study, no cohesive resin fractures were observed. Oxidizing substances, such as ozone, hydrogen peroxide, and carbamide peroxide, which remain on the surface of dental tissues, can negatively affect the monomer chains that grow during adhesive polymerization (Mirzaei *et al.*, 2013; Rodrigues *et al.*, 2011). The studies by Qeblawi *et al.*(2015); Arslan *et al.* (2011); Ahn *et al.* (2020); and Qeblawi *et al.* (2015) suggest that in shear strength tests, the distribution of stresses in the bonding 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 observed in this study, particularly in the incisal and middle thirds (Figure 2). The oxidizing action of ozone may interfere with the prismatic structure of the dental substrate, reducing the neutralization of acidic monomers. This, in turn, affects the setting reaction and polymerization of the materials (Ahn *et al.*, 2020). The non-neutralization of monomers can increase water sorption, solubility, and hygroscopic expansion stress (Vrochari *et al.*, 2010), which may explain the pattern of cohesive fractures in resin.

When administering ozone, caution is necessary for both the professional and the patient, as the inhalation of ozone can be toxic to the pulmonary system and other organs (Manso & Carvalho, 2017). Additionally, the complex preparation process and limited usage time may present further challenges in this area (Naik *et al.*, 2016).

Given the limitations of this study, further laboratory tests and clinical trials are necessary to confirm the data obtained in this in vitro study.

The literature with studies showing bond strength after the use of ozonized water is still very limited, especially concerning the longevity of restorative procedures following ozone application. A suggestion for future research would be studies evaluating bond strength over longer periods, such as 6 months and 1 year. Additionally, it is needed to establish optimal protocols for ozone application.

Conclusion

Ozonated water demonstrated superior bond strength on enamel in the cervical third after 24 hours of storage. However, no statistically significant differences in bond strength were observed in other storage periods or tooth regions (middle and incisal thirds). This suggests that while ozonated water may enhance early bond performance in specific areas, its long-term effects and influence on other tooth regions appear comparable to those of distilled water.

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Author's Contributions

Fernanda Rafaela Ribeiro: Concept and design, acquisition, analysis, or interpretation of data, drafting of the manuscript.

Caterine Renata Passaglia: Acquisition, analysis, or interpretation of data.

Marcio José Mendonça and Julio Katuhide Ueda: Acquisition, analysis, or interpretation of data, Critical review of the manuscript for important intellectual content; supervision.

Veridiana Camilotti: Conception and design, acquisition, analysis, or interpretation of data, critical review of the manuscript for important intellectual content; supervision.

Ethics

The authors state that this study complies with and respects scientific ethics.

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